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# 부신피질 호산성 과립세포종 중 불확실한 악성 잠재력 및 종양세포종 아형 환자들의 불량한 예후를 예측하기 위한 위험인자 분석

Risk Factor Analysis to Predict Poor Prognosis in Patients having Uncertain Malignant Potential and Oncocytoma Subtypes within Oncocytic Adrenocortical Neoplasm

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김 병 창

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이 논문을 의학박사 학위 논문으로 제출함

2024 년　2 월

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**ABSTRACT**

**Background**: Oncocytic adrenocortical neoplasms (OANs) are extremely rare adrenal tumors, and their diagnosis is challenging. The aim of this study was to conduct a risk factor analysis to predict poor prognosis in patients diagnosed with an oncocytic adrenocortical neoplasm of uncertain malignant potential (OANUMP) and oncocytoma, both OAN subtypes.

**Materials and Methods**: We retrospectively analyzed 20 patients diagnosed with OANUMP or oncocytoma after adrenalectomy from February 2002 to May 2022. Six patients pathologically re-classified after pathology review as oncocytic adrenocortical carcinoma (OAC) were excluded. Fourteen eligible patients were divided into two groups according to recurrence status. We compared the clinicopathological and radiological features of the two groups. Especially, we analyzed the computed tomography (CT) scan features and examined the pathological features related to malignant lesions.

**Results**: Among the 14 patients, recurrence occurred in 3 (21%; 2 [67%] and 1 [33%] patients with OANUMP and oncocytoma, respectively). The recurrence group had a higher proportion of patients with pathological necrosis than the no evidence of disease (NED) group (66.7% *vs.* 9.1%, $p$ =0.031), along with a higher proportion of patients with a Helsinki score >8.5 (66.7% *vs.* 9.1%, $p$ =0.031). The recurrence group had a higher proportion of patients with an indeterminate pathological resection margin (100% *vs.* 63.6%, $p$ =0.051), malignant features on CT scan, peri-adrenal soft tissue extension on pathology, and adrenocortical malignancy according to the reticulin algorithm than the NED group. Expression of p53 and Ki-67 and the proportion of β-catenin-positive patients did not differ significantly between the two groups.

**Conclusion**: In patients with OANUMP or oncocytoma, the presence of pathological necrosis, Helsinki score >8.5, and indeterminate pathological resection margin were highly associated with recurrence in univariate analysis. The presence of peri-adrenal soft tissue extension, malignant features on CT scan, and adrenocortical malignancy according to the reticulin algorithm were associated with the potential recurrence. These factors could be risk factors for poor prognosis, warranting a short-term, regular follow-up of patients with OANUMP or oncocytoma within OAN.

**Keywords**: OAN, OANUMP, Oncocytoma, Adrenalectomy, Helsinki score, Reticulin algorithm

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## Introduction

Oncocytic adrenocortical neoplasms (OANs) are very unusual variants of adrenocortical tumors. OANs exclusively or predominantly comprise oncocytes, large polygonal cells characterized by granular eosinophilic cytoplasm owing to an accumulation of abnormal mitochondria [1]. Although occasionally found in the thyroid, kidney, and salivary glands, these tumors are notably rare in the adrenal cortex. Typically, OANs are considered nonfunctioning and benign and are known to exhibit a larger size than other adrenal tumor variants [2]. According to the Lin-Weiss-Bisceglia (LWB) criteria proposed in 2004, OANs can be classified into oncocytic adrenocortical carcinoma (OAC), oncocytic adrenocortical neoplasm of uncertain malignant potential (OANUMP), and benign oncocytoma, and recent studies have indicated the potential hormonal functionality of OANs, including subclinical Cushing syndrome, cortisol excess, and androgen excess [3,4,5].

The standard therapy for OANs mainly relies on adrenalectomy, given that OANs typically present as a large adrenal mass. Laparoscopic adrenalectomy is more widely performed than conventional open adrenalectomy owing to its lower morbidity and shorter duration of hospitalization [6,7]. Laparoscopic adrenalectomy is undertaken assuming the absence of capsular or vascular invasion and invasion of surrounding structures, as well as the ability to achieve complete resection without disrupting the capsule during surgery. Moreover, adrenalectomy using robotic systems for OANs has been reported recently [8].

The LWB criteria are divided into two categories, with major criteria including mitoses > 5 per 50 high-power fields (HPF), atypical mitosis, and venous invasion, and minor criteria including large size (>10cm), necrosis, capsular invasion, and sinusoidal invasion. OANs with one or more major criteria are classified as OAC; those with one or more minor criteria are classified as OANUMP; and tumors without both major and minor criteria are classified as oncocytoma [3]. According to a recent systemic review focused on OANs, the overall survival rates for OAC, OANUMP, and oncocytoma were 47,88, and 100%, respectively, with a median follow-up duration of 24 months. The authors found that of the 89 patients with OANs, 14 experienced recurrences, among whom one was diagnosed with oncocytoma [9].

Currently, factors influencing the aggressiveness of OANUMP remain poorly explored. In a previous report, a patient initially diagnosed with oncocytoma of low-grade malignant potential post adrenalectomy and expected to have a favorable postoperative prognosis was subsequently diagnosed with bone and liver metastasis [10]. Given the markedly rare incidence of OANs, including OANUMP and oncocytoma, follow-up studies presenting the long-term prognosis of such neoplasm remain scarce.

This study was to analyze the patients pathologically confirmed to have OANUMP and oncocytoma after adrenalectomy, aiming to determine the risk factors associated with predicting a poor prognosis.

## Materials and Methods

### *Patients and data collection*

This retrospective study involved patients who underwent adrenalectomy for adrenal incidentaloma from February 2000 to May 2022 at a tertiary medical center. In total, 760 patients were diagnosed with nonfunctioning adrenal incidentaloma, among whom 143 patients underwent adrenalectomy during the study period. In the initial pathological reports, 26 patients were categorized as having OANs, and among them, 13 patients were diagnosed with OANUMP, and 7 patients were diagnosed with oncocytoma. Six patients diagnosed with OAC were excluded from the current study. The pathology slides of 20 patients, including patients with OANUMP and oncocytoma, were reviewed by a single experienced endocrine pathologist. In the revised pathology reports, 6 patients were re-classified as OAC, 9 as OANUMP, and 5 as oncocytoma. Finally, 14 patients were included in the current study for further evaluation (Figure 1).

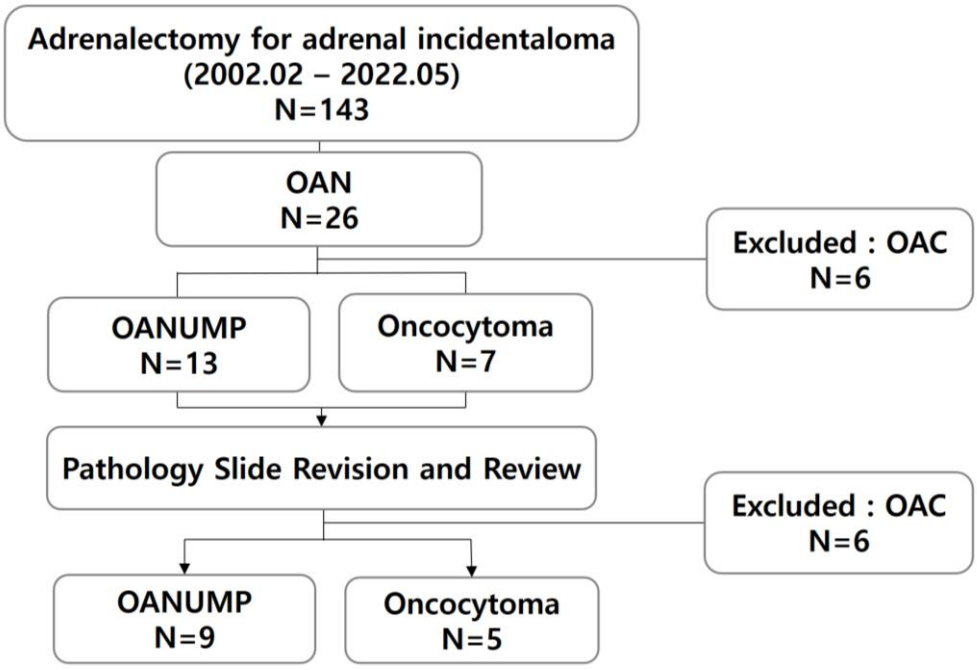


**Fig.1** Flowchart of study population

**Abbreviations:** OAN, oncocytic adrenocortical neoplasm; OANUMP, oncocytic adrenocortical neoplasm of uncertain malignant potential; OAC, oncocytic adrenocortical carcinoma

Subsequently, the patients were divided into two groups according to their recurrence status. Recurrence was defined as the tumor reoccurring in the same place (local recurrence), nearby (regional recurrence), or in another location (distant recurrence) after the initial adrenalectomy. Follow-up assessments included computed tomography (CT), magnetic resonance imaging, and/or position emission tomography imaging, performed every 3–6 months postoperatively. The patients' data included demographics such as age, sex, weight, height, and body mass index (BMI), preoperative CT scan features (e.g., tumor size, Hounsfield unit (HU), and washout %), operative factors (e.g., operation type and resection margin status), and postoperative pathological features (e.g., tumor size, resection margin status, histochemical features, and immunohistochemical features). This retrospective study was approved by the Institutional Review Board of our institution (No. 2022-0976), and the need for informed consent from patients was waived owing to the retrospective nature of the study.

*Preoperative CT scan findings*

Contrast-enhanced adrenal CT or abdominal CT scans were performed, and the washout of intravenous contrast medium was calculated at 60 to 90 s (portal venous phase) and at 10–15 min (delayed enhancement phase) after contrast administration. To calculate the washout of intravenous contrast medium for the tumor, HU was measured during the precontrast, portal venous, and delayed phases. Absolute washout was calculated as the difference between the attenuation value in the HU in an early enhanced CT and the HU on a delayed CT image. This difference was divided by the HU difference between the early enhanced CT and an unenhanced CT image, with the result then multiplied by 100%. Relative washout was calculated by subtracting the HU on a delayed CT image from that on an early enhanced CT image. The resulting value was divided by the HU on the enhanced CT image and then multiplied by 100% [11]. The adrenal tumor size was measured by identifying the longest tumor diameter of the tumor on the image displaying the largest lesion area in the cross-sectional view of the CT scan. Malignant features on the CT scan were defined as tumor size >4cm, precontrast HU >10, absolute washout <60%, and relative washout <40% [12]. Figure 2 represents an example of tumor size and HU measurements.

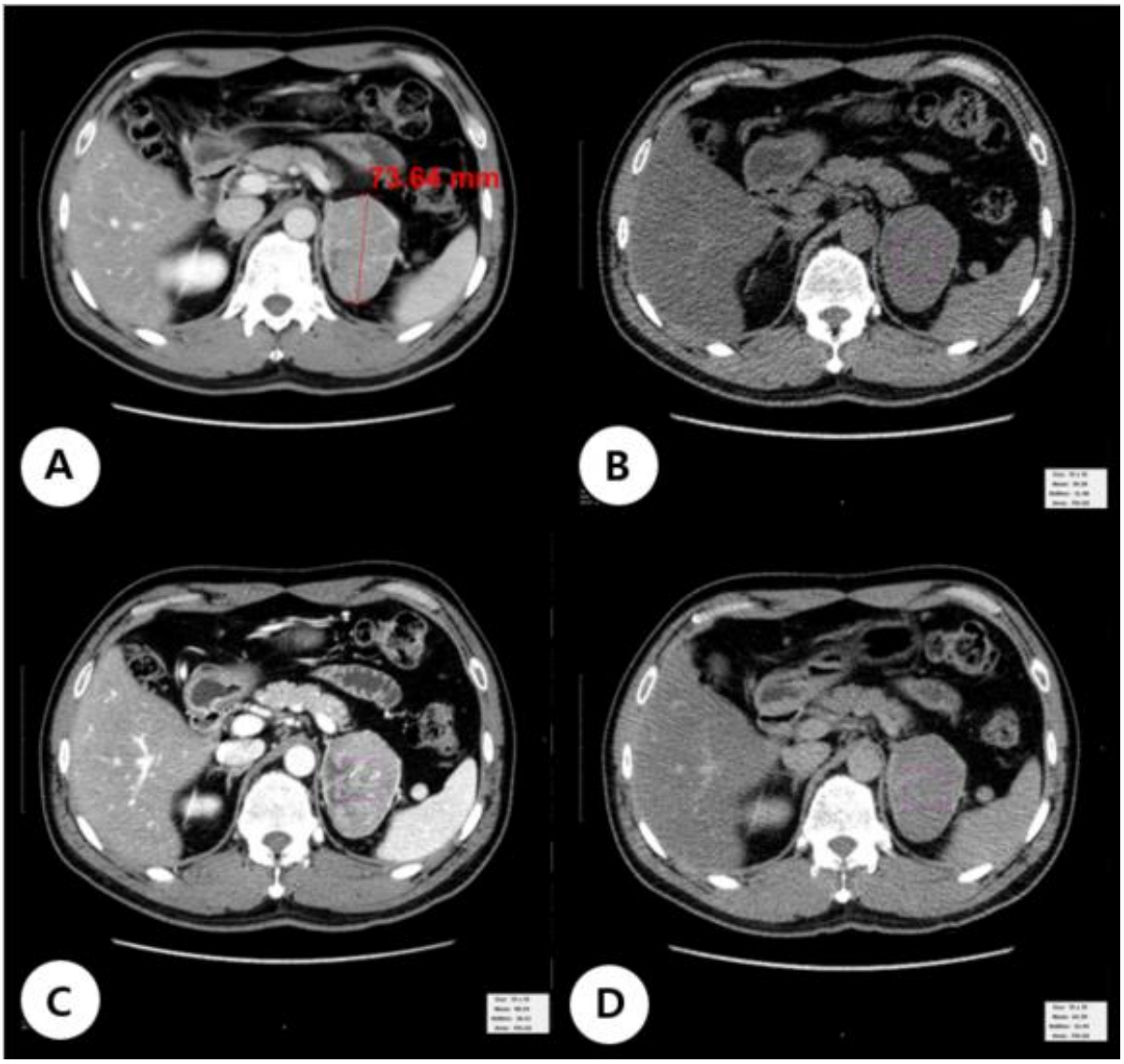


**Fig.2** Tumor size and Hounsfield unit (HU) measurement on computed tomography (CT) scan. (A) measurement of adrenal tumor size; (B) measurement of HU in unenhanced CT; (C) measurement of HU in portal venous phase (1 min) CT; (D) measurement of HU in delayed phase (15 min) CT.

*Surgical techniques and Intraoperative findings*

Laparoscopic and robotic retroperitoneal posterior adrenalectomy, laparoscopic transperitoneal adrenalectomy, and conventional open adrenalectomy were performed. Among the 14 patients, one, three, eight, and two patients underwent conventional open adrenalectomy, laparoscopic transperitoneal adrenalectomy, laparoscopic retroperitoneal adrenalectomy, robotic retroperitoneal adrenalectomy, respectively. During the surgical procedure, the status of the gross resection margin was assessed by

examining the edges or borders of the excised tissue. The gross resection margin was established using the intraoperatively identified margin through either instrumental inspection via telescope view or the palpation of operative field during open adrenalectomy. In addition to the negative or positive margin safety of the tumor, an unknown gross margin refers to no record of margin safety in the clinical data. For the pathological resection margin, the indeterminate margin included intraoperatively fragmented specimens within the specimen retrieval bag when the negativity or positivity of the actual margin could not be confirmed.

*Samples and postoperative pathological evaluation*

Paraffin-embedded blocks from 20 patients (13 OANUMPs and 7 oncocytomas) based on the initial pathology reports were utilized for the evaluation. In all cases examined, tissue sections measuring 4-μm thick tissue sections were obtained from a representative paraffin-embedded sample. All surgical samples were reviewed by an experienced pathologist blinded to clinical histories or outcomes. All adrenal tumors were reviewed according to the LWB criteria and classified into three subtypes. The mitotic grade was determined by counting mitotic figures in 50 high-power fields or 10 $mm^2$ from areas with high mitotic density in all tumor samples. Figure 3 presents a representative pathology image of OANs.

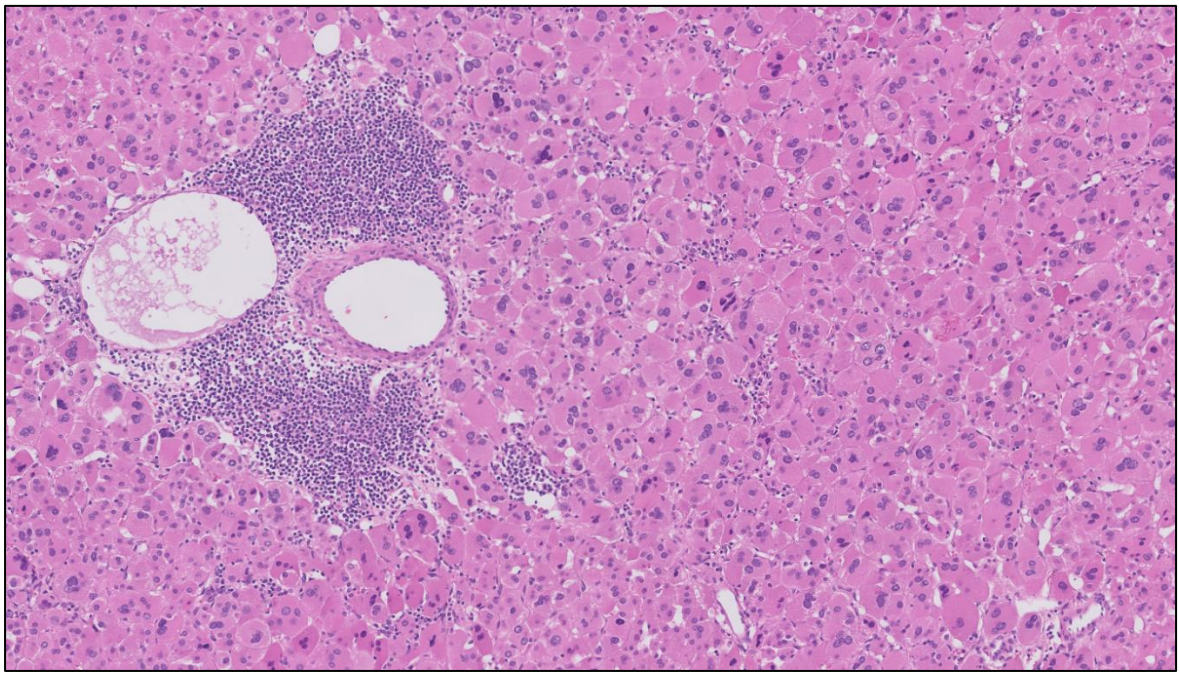

**Fig.3** Oncocytic adrenocortical neoplasm. Diffuse proliferation of large polygonal cells with abundant eosinophilic cytoplasm and occasional enlarged atypical nuclei on Hematoxylin-Eosin stain.

*Histochemistry and Immunohistochemistry*

Representative markers related to the aggressiveness of adrenocortical neoplasm were selected for staining based on previous studies [13]. Hematoxylin-eosin (HE) staining was performed to confirm the diagnosis of OAN and monoclonal antibody staining was performed against Ki-67 (mouse clone MIB-1, Dako), p53 (mouse clone DO-7, Dako), β-catenin (mouse clone 14, Cell Marque), and phospho-histone H3 (PHH3) (rabbit clone POLY, Cell Marque). Table 1 presents detailed information regarding the antibodies employed.

**Table 1.** Detailed information regarding monoclonal antibodies used in the immunohistochemical stains

| Antibody | Manufacturer | Species | Clone | Dilution |
|---|---|---|---|---|
| Ki-67 | Dako | Mouse | MIB1 | 1:200 |
| p53 | Dako | Mouse | DO-7 | 1:1000 |
| β-catenin | Cell Marque | Mouse | 14 | 1:200 |
| PHH3 | Cell Marque | Rabbit | POLY | 1:200 |

**Abbreviation:** PHH3, phospho-histone H3.

Immunohistochemical staining was performed using an automated stainer (BenchMark XT automatic immunostaining device by Ventana), in accordance with the manufacturer's recommendations. Sections were placed on silanized charged slides, dried, and then incubated. Cell Conditioning 1 (CC1) buffer and autoimmunostrainer were utilized for epitope retrieval and antigen-antibody reactions, respectively. Consequently, slides were counterstained and prepared for microscopic analysis.

The expression of p53 was evaluated based on the overall production of positive nuclear or cytoplasmic staining and was classified into four categories: 0,1,2, and 3, corresponding to <5, 33, 66, and >66% positivity, respectively. Ki-67 was determined by manually calculating the percentage of tumor cells exhibiting positive nuclear staining out of 1,000-2,000 tumor cells in the hotspot area. The antibody expression ratios were compared and analyzed categorically, indicating either positive expression or loss of antibody expression. Specimens exhibiting only membranous staining were deemed negative for β-catenin expression, whereas those with cytoplasmic or nuclear staining were classified as positive for β-catenin expression. Mitotic figures were counted in 50 successive HPF on HE slides. Atypical mitoses were evaluated in HE or slides stained with PHH3. In these stained sections, mitotic figures and atypical mitoses were identified based on positive staining and specific morphological characteristics.

The reticulin framework was examined using Gomori's silver impregnation method. An altered reticulin network was defined as reticulin fibrils with variable, irregular thickness with a frayed appearance surrounding single or small groups of cells (qualitative alteration) and loss of reticulin framework (quantitative alteration). A normal reticulin network was defined as intact when reticulin fibrils of the same thickness completely surrounded the adrenal cortical cells in nests and cords [14]. The present study primarily evaluated the loss of the reticulin framework.

*Diagnostic algorism and scoring system of OANs*

In addition to the LWB criteria-based classification into OAC, OANUMP, and oncocytoma, the aggressiveness of OANs was assessed using the reticulin algorithm and the Helsinki scoring system. In the reticulin algorithm, malignancy is defined as an altered reticulin framework associated with one of

the following three parameters: necrosis, high mitotic rate, and vascular invasion [14,16,17]. Helsinki score was calculated as follows: 3 × mitotic rate (>5/50 HPF) + 5 × presence of necrosis + proliferation index in the most proliferative area of the tumor. The aggressiveness of the neoplasm was classified based on previous studies: Helsinki score 0–8.5, Helsinki score 8.5–17, and Helsinki score > 17 [17,18].

*Statistical analysis*

Data analyses were performed using SPSS statistics version 26.0 (IBM Corp., Armonk, NY). Continuous variables, reported as median (minimum-maximum), were compared using the Mann–Whitney U test. Categorical variables were examined using the Fisher exact test and $\chi 2$ test. All $p$ values <0.05 were considered statistically significant.

**Results**

*Changes in diagnosis before and after retrospective pathology review*

Figure 4 presents a flowchart depicting the changes in diagnosis before and after the review of the pathology. Out of 13 patients who were initially diagnosed with OANUMPs, recurrence was observed in 4 patients, while 9 patients remained recurrence-free. Among the 7 patients who were initially diagnosed with oncocytomas, recurrence was observed only in 1 patient, while 6 patients remained recurrence-free. After the pathology review, among the 4 patients with recurrent OANUMP, 2 were re-classified as OAC, whereas among the 9 nonrecurrent patients with OANUMP, 3 were re-classified as OAC. Following pathological review, the initial diagnosis was retained in one patient with recurrent oncocytoma, while among the 6 nonrecurrent patients with oncocytoma, one was re-classified as OAC and one as OANUMP.

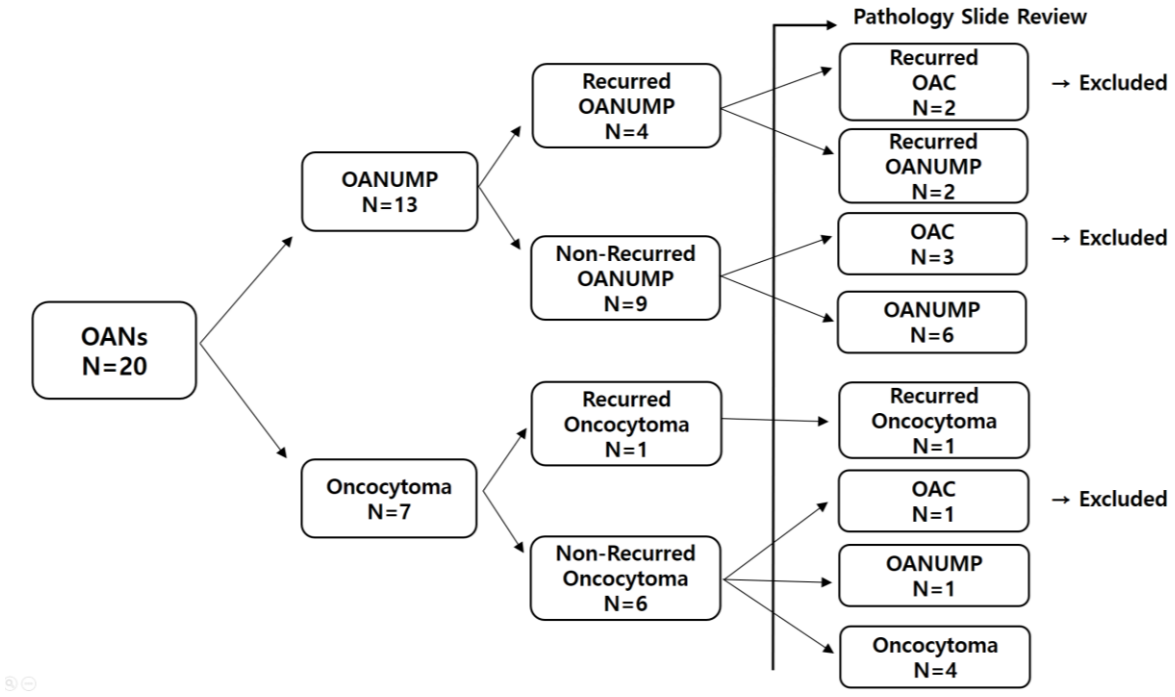


**Fig.4** Flowchart depicting the changes in diagnosis before and after pathologic review

**Abbreviations:** OAN, oncocytic adrenocortical neoplasm; OANUMP, oncocytic adrenocortical neoplasm of uncertain malignant potential; OAC, oncocytic adrenocortical carcinoma.

*Baseline clinical characteristics*

Table 2 summarizes the clinical characteristics of the 14 patients with OANUMP or oncocytoma according to the recurrence. No significant differences in age, sex, height, weight, BMI, sites of the tumor, and types of operation were observed between the recurrence and NED groups. The tumor gross resection margins, confirmed during the surgery, were clear in all patients.

*Preoperative CT scan features*

Table 2 summarizes the preoperative CT scan features of the 14 patients. The recurrence group displayed a slightly larger median tumor size than the NED group, although no statistically significant difference was observed (6.6 cm *vs*. 5.5 cm, $p$ =0.102). Median precontrast HU values exceeded 10 in both groups, with almost no difference between the two groups (36.5 *vs*. 32.7, $p$ =0.999). The recurrence group has lower median values of absolute washout (37.2% *vs*. 64.1%, $p$ =0.166) and relative washout

(28.1% *vs*. 45.6%, $p$ =0.405) than the NED group, where values exceeded 60% and 40% for absolute and relative washout, respectively. Moreover, the recurrence group had a higher proportion of patients exhibiting three malignant features than the NED group, although no statistically significant difference was observed (66.7% *vs*. 22.2%, $p$ =0.236).

*Postoperative pathology features*

Table 3 summarizes the postoperative pathological characteristics of 14 patients with OANUMP or oncocytoma according to the recurrence. The recurrence group had a larger median tumor size than the NED group, although no statistically significant difference was detected (7.3 cm *vs*. 4.8 cm, $p$ =0.368). The recurrence group had a higher proportion of patients exhibiting pathological necrosis than the NED group, accompanied by a significant statistical difference (66% *vs*. 9%, $p$ =0.031). The recurrence group comprised a higher proportion of patients exhibiting microscopic sinusoidal and capsular invasion on HE staining than the NED group, although there was no statistical difference.

**Table 2.** Clinical features of patients with oncocytic adrenocortical neoplasm of uncertain malignant potential and oncocytoma according to the recurrence

| Variables | Total (N=14) | Recurrence group (*N* = 3) | NED group (*N* = 11) | *p* value |
|---|---|---|---|---|
| Age (years) | 47(41-80) | 47(42-59) | 47(41-80) | 0.937 |
| Sex | | | | 0.923 |
| Male | 9(64.2) | 2(66.7) | 7(63.6) | |
| Female | 5(35.8) | 1(33.3) | 4(36.4) | |
| Height (cm) | 160(149.9-172.6) | 159.5(158.8-172.6) | 160.5(149.9-171.7) | 0.484 |
| Weight (kg) | 65.9(47.3-85.5) | 63.2(58.9-85.5) | 68.7(47.3-82.2) | 0.484 |
| BMI (kg/m2) | 25.0(19.0-29.4) | 25.0(23.1-28.7) | 25.1(19.0-29.4) | 0.938 |
| Diagnosis | | | | 0.923 |
| OANUMP | 9(64.2) | 2(66.7) | 7(63.7) | |
| Oncocytoma | 5(35.8) | 1(33.3) | 4(36.3) | |
| Size of tumor in CT (cm) | 5.6(2.0-20) | 6.7(6.2-7.2) | 5.5(2.0-20) | 0.102 |
| Site of tumor | | | | 0.999 |
| Right | 7(50) | 2(66.7) | 5(45.5) | |
| Left | 7(50) | 1(33.3) | 6(54.5) | |
| Precontrast HU | 32.7(23.7-49.4) | 36.5(23.7-49.4) | 32.7(24.7-40.5) | 0.999 |
| Absolute washout (%) | 62.6(12-80) | 37.2(12-63) | 64.1(13-80) | 0.166 |
| Relative washout (%) | 45.6(5-57) | 28.1(5-51) | 45.6(8-57) | 0.405 |
| Malignant feature on CT | | | | 0.236 |
| Yes | 4(35.8) | 2(66.7) | 2(22.2) | |
| No | 9(64.2) | 1(33.3) | 7(77.8) | |
| Operation type | | | | 0.999 |
| Open | 1(7.3) | 0 | 1(9.1) | |
| Laparoscopic transperitoneal | 3(21.4) | 1(33.3) | 2(18.2) | |
| Laparoscopic retroperitoneal | 8(57.1) | 2(66.7) | 6(54.5) | |
| Robot retroperitoneal | 2(14.2) | 0 | 2(18.2) | |
| Gross resection margin | | | | N/A |
| Tumor negative | 14(100) | 3(100) | 11(100) | |
| Indeterminate | 0 | 0 | 0 | |

**Abbreviation:** BMI, body mass index; CT, computed tomography; HU, hounsfield unit; NED, No Evidence Of Disease; OANUMP, oncocytic adrenocortical neoplasm of uncertain malignant potential

**Table 3.** Pathological features of patients with oncocytic adrenocortical neoplasm of uncertain malignant potential and oncocytoma according to the recurrence

| Variables | Total (N=14) | Recurrence group (*N* = 3) | NED group (*N* = 11) | *p* value |
|---|---|---|---|---|
| Size of tumor(cm) | 5.7(2.5-23.5) | 7.3(5.5-7.5) | 4.8(2.5-23.5) | 0.368 |
| Necrosis | | | | 0.031 |
| Yes | 3(21.5) | 2(66.7) | 1(9.1) | |
| No | 11(78.5) | 1(33.3) | 10(90.9) | |
| Capsular invasion | | | | 0.347 |
| Yes | 6(42.9) | 2(66.7) | 4(36.4) | |
| No | 8(57.1) | 1(33.3) | 7(63.6) | |
| Sinusoidal invasion | | | | 0.347 |
| Yes | 6(42.9) | 2(66.7) | 4(36.4) | |
| No | 8(57.1) | 1(33.3) | 7(63.6) | |
| Margin | | | | 0.051 |
| Tumor negative | 4(28.6) | 0 | 4(36.4) | |
| Indeterminate | 10(71.4) | 3(100) | 7(63.6) | |
| Soft tissue extension | | | | 0.396 |
| Yes | 2(14.3) | 1(33.3) | 1(9.1) | |
| No | 12(85.7) | 2(66.7) | 10(90.9) | |
| Reticulin alteration | | | | 0.515 |
| Yes | 7(50) | 2(66.7) | 5(45.5) | |
| No | 7(50) | 1(33.3) | 6(54.5) | |
| Atypical mitosis on PHH3 stain | | | | N/A |
| Yes | 0 | 0 | 0 | |
| No | 14(100) | 3(100) | 11(100) | |
| β-catenin | | | | 0.707 |
| Positive | 6(42.9) | 1(33.3) | 5(45.5) | |
| Negative | 8(57.1) | 2(66.7) | 6(54.5) | |
| P53 | | | | N/A |
| 0(<5%) | 0 | 0 | 0 | |
| 1(5–33%) | 14(100) | 3(100) | 11(100) | |
| 2(33–66%) | 0 | 0 | 0 | |
| 3(>66%, overexpression) | 0 | 0 | 0 | |

| | | | | |
|---|---|---|---|---|
| Mitotic figure/ 10mm2 | 1.0(0-3) | 1.0(0-3) | 1.0(0-3) | 0.695 |
| Ki-67 labeling index (%) | 1.4(0.5-5.9) | 1.2(0.9-2.9) | 1.6(0.5-5.9) | 0.738 |
| Helsinki score | | | | 0.031 |
| 0–8.5 | 11(78.6) | 1(33.3) | 10(90.9) | |
| 8.5–17 | 3(21.4) | 2(66.7) | 1(9.1) | |
| <17 | 0 | 0 | 0 | |
| Reticulin algorithm | | | | 0.093 |
| Malignant | 3(21.5) | 2(66.7) | 1(9.1) | |
| Not malignant | 11(78.5) | 1(33.3) | 10(90.9) | |
| Minor score (LWB criteria) | 1.0(0-3) | 3.0(0-3) | 1.0(0-3) | 0.368 |

**Abbreviation:** LWB criteria, Lin-Weiss-Bisceglia criteria; PHH3, phospho-histone H3

Figures 5(A), 5(B), and 5(C) represent examples of pathological features of patients exhibiting capsular invasion, sinusoidal invasion, and necrosis, respectively. All patients in the recurrence group and 63.6% of patients in the NED group showed indeterminate margins, and this difference was marginally significant ($p$ =0.051). The proportion of patients with peri-adrenal soft tissue extension was higher in the recurrence group than that in the NED group (66% *vs.* 9%, $p$ =0.396). There were no significant differences in the proportion of patients exhibiting reticulin alteration and β-catenin positive expression in pathology. Figures 5(E) and 5(F) represent examples of pathological features of patients exhibiting peri-adrenal soft tissue extension and reticulin alteration, respectively. Both groups had similar mitotic figure counts, partly confirmed using PHH3 as a mitosis marker, and no patient showed atypical mitosis. Figure 5(D) illustrates an example of the pathological features of atypical mitosis.

Considering p53 expression, all patients in both groups were classified as category 1 (5–33%). The median Ki-67 labeling index did not differ significantly between both groups (1.2 *vs*. 1.6, $p$ =0.738).

The recurrence group had a higher proportion of patients with a Helsinki score of 8.5–17 than the NED group, and this difference was statistically significant (66.7% *vs*. 9.1%, $p$ =0.031). No patients exhibited a Helsinki score >17. Additionally, the recurrence group had a higher proportion of patients with malignancy according to the reticulin algorithm than the NED group, although the difference was not statistically significant difference (66.7% *vs*. 9.1%, $p$ =0.368).

Table 4 summarizes the detailed characteristics regarding recurrence, treatment, histopathological, and

radiological features of 3 recurred patients. Figure 6 presents a summary comparing histopathological and radiological features between patients in the recurrence and NED groups.

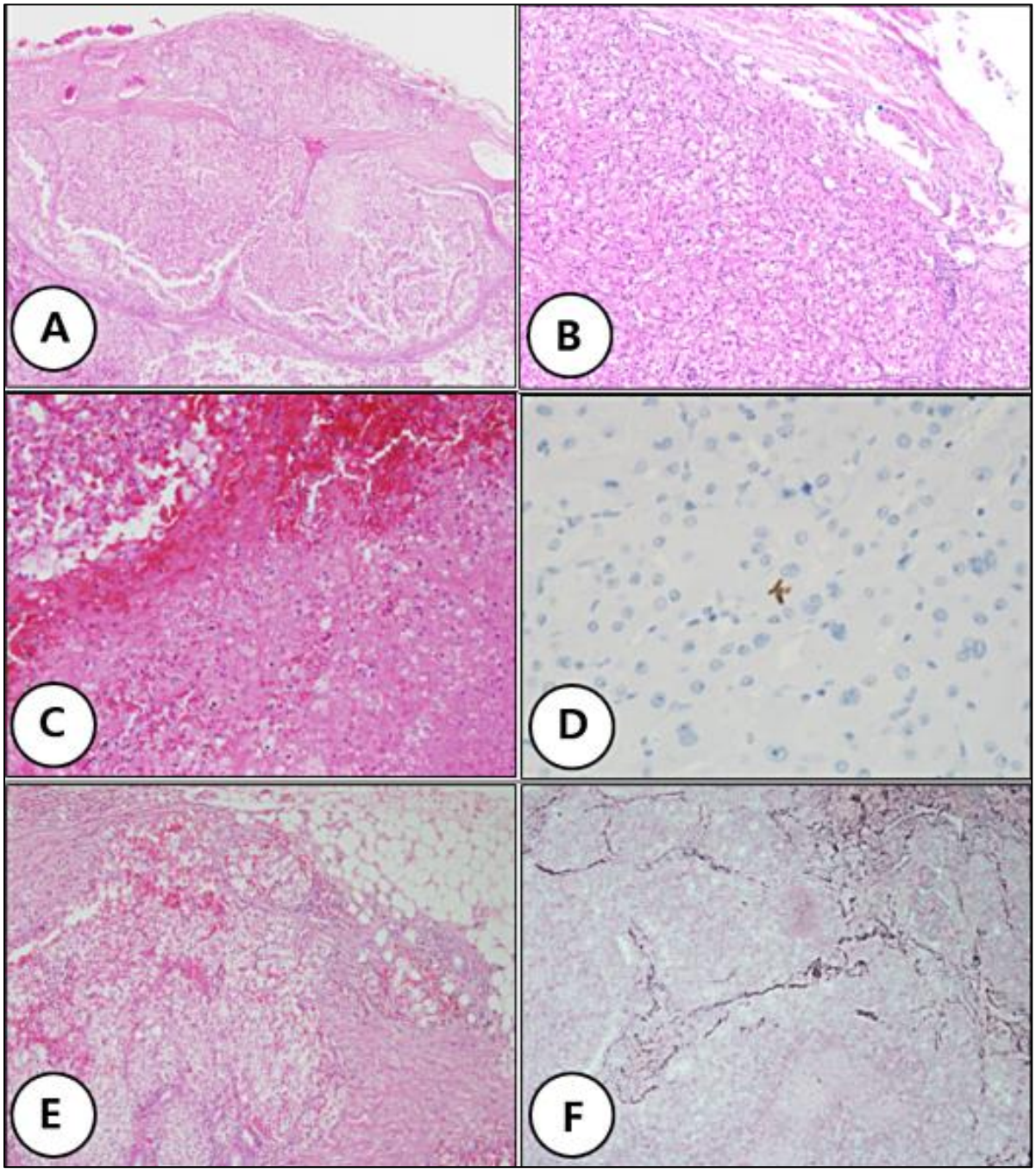


**Fig.5** Histopathological features of oncocytic adrenocortical neoplasm. (A) capsular invasion (40×, Hematoxylin-eosin [HE] stain); (B) sinusoidal invasion (100×, HE stain); (C) necrosis (100×, HE stain); (D) atypical mitosis (400×, PHH3); (E) peri-adrenal soft tissue extension (100×, HE stain); (F) reticulin alteration (100×, Gomori's silver impregnation)

**Table 4.** Patient characteristics with recurrence after adrenalectomy

| Patient number | Age/Sex | Operation date | Initial diagnosis | 1st recurrence (month) | Last follow-up date | Recurred site (1st – 2nd) | Treatment methods | Final diagnosis |
|---|---|---|---|---|---|---|---|---|
| 1 | 59/F | 2014.07.11 | OANUMP | 5.4 | 2015.12.08 | Operated bed (peritoneal seeding) | Mitotane | OANUMP |
| 2 | 47/M | 2017.09.22 | OANUMP | 7.1 | 2022.11.28 | 1st lung (Left)<br>2nd lung (Right) | Resection, Mitotane | OANUMP |
| 3 | 42/F | 2018.03.09 | oncocytoma | 23.9 | 2022.11.25 | Operated bed | Resection | oncocytoma |

| Patient number | Tumor size on CT (cm) | Malignant features on CT | Indeterminate pathological margin | Pathological necrosis / capsular invasion / sinusoidal invasion | Peri-adrenal soft tissue extension | Reticulin alteration | Helsinki score >8.5 | Reticulin algorithm (malignant) |
|---|---|---|---|---|---|---|---|---|
| 1 | 6.7 | + | + | +/+/+ | + | + | + | + |
| 2 | 6.2 | + | + | +/+/+ | - | + | + | + |
| 3 | 7.2 | - | + | -/-/- | - | - | - | - |

**Abbreviation:** OANUMP, oncocytic adrenocortical neoplasm of uncertain malignant potential; CT, computed tomography

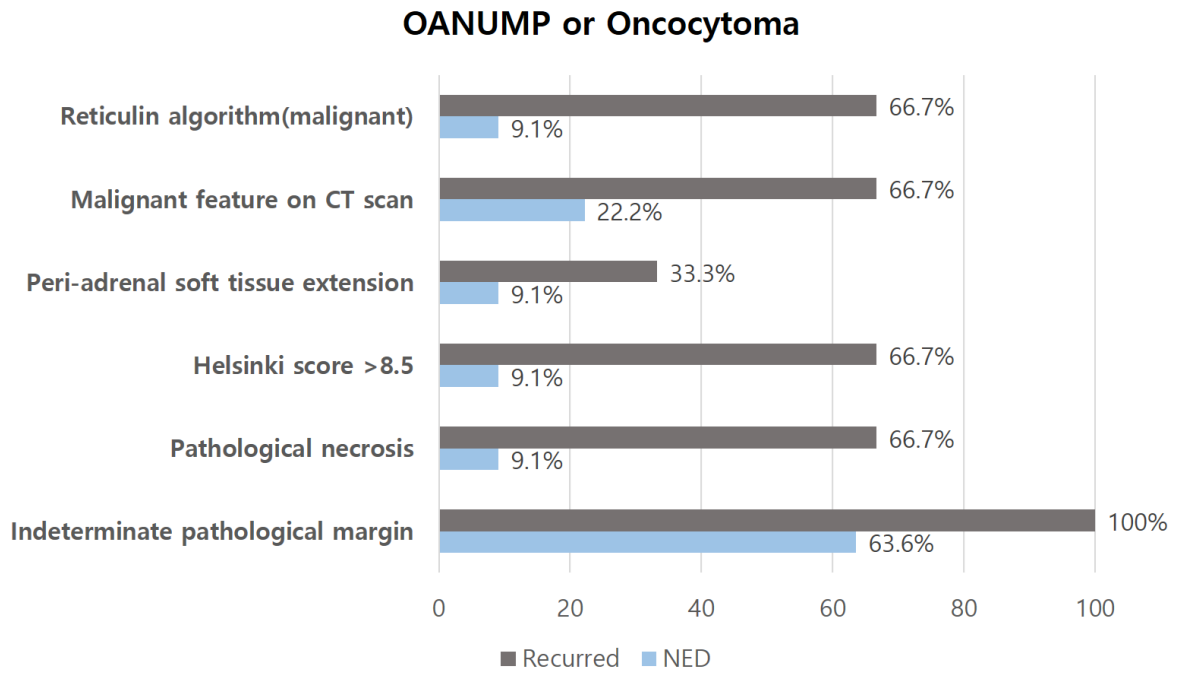


**Fig.6** Comparison of histopathologic and radiologic features between the recurrence and no NED groups in patients with oncocytic adrenocortical neoplasm of uncertain malignant potential (OANUMP) or oncocytoma based on the Lin-Weiss-Bisceglia criteria.

**Abbreviation:** CT, computed tomography; NED, no evidence of disease; OANUMP, oncocytic adrenocortical neoplasm of uncertain malignant potential

## Discussion

In the present study, we evaluated the risk factors associated with poor prognosis in patients diagnosed with OANUMP and oncocytoma after adrenalectomy. We found that patients with recurrent OANUMP or oncocytoma had absolute and relative washout values less than 60 and 40% of adrenal tumors on CT scans, respectively; these values were lower than those in patients without recurrence. Furthermore, the recurrence group comprised a higher proportion of patients exhibiting a combination of three factors (tumor size >4 cm, precontrast HU >10, and absolute washout <60% with relative washout <40%) indicative of malignant features than the group with recurrence. Moreover, a higher proportion of patients with recurrence exhibited necrosis and peri-adrenal soft tissue extension in the pathological review. Additionally, a higher proportion of patients with recurrence had a Helsinki score >8 than those without recurrence. Likewise, the recurrence group comprised a higher proportion of patients with malignancy according to the reticulin algorithm than the group without recurrence. All patients with recurrence exhibited indeterminate margins.

Juliano et al. have reported a change in the diagnosis in the OAN subtype in one patient [10]. The patients initially diagnosed with oncocytoma of low-grade malignant potential after adrenalectomy exhibited disturbing malignant potential upon the secondary pathological review. Subsequently, the patient developed liver and bone metastasis. In addition, Huang et al. have reported a case of a patient initially diagnosed with OANUMP, which was later confirmed as OAC with concomitant scalp and lung metastases [19]. In the present study, among the 20 patients initially diagnosed with OANUMP or oncocytoma, the diagnosis of 7 (35%) patients was altered following a pathological review, and out of these patients, 2 experienced recurrences. These findings clearly indicate the challenges associated with the pathological diagnosis of OAN subtypes. Moreover, our results imply that even the currently used LWB criteria have limitations in terms of classifying OAN subtypes.

According to the LWB criteria, evidence indicative of malignant OANs includes atypical mitosis, mitoses >5/50 HPF, and venous invasion. In the present study, following pathological review, all six patients who were re-classified as OAC and subsequently excluded exhibited atypical mitosis. Among

them, recurrence was observed in two cases. However, patients diagnosed as OANUMP according to the LWB criteria without atypical mitosis also exhibited malignant features, including recurrence. Accordingly, reassessing the discriminatory power of the LWB criteria for predicting malignancy needs to be performed in future investigations.

In previous reports, radiological features of OANs have been scarcely described, with the majority of patients exhibiting a size >4 cm on CT scans [9,20,21]. In 2021, a treatment algorithm and imaging features for patients with adrenal incidentalomas were published. On the CT scan, adrenal tumors >4 cm in size, precontrast HU >10, and contrast washout <40–60% were described as displaying a suspicious malignant appearance [12]. Khan et al. have reported that malignant OANs, similar to adrenocortical carcinoma (ACC), present an absolute washout of <60% and a relative washout of <40% [22]. In the present study, a significantly higher proportion of patients with recurrence satisfied all three of these radiological suspicious malignant factors than the proportion of patients without recurrence. Accordingly, three of the suspicious malignant factors on CT scans indicate a potential risk of recurrence among patients diagnosed with OANUMP or oncocytoma.

The presence of microscopic tumor necrosis and involvement of microscopic tumor margin are documented factors associated with the aggressiveness of adrenocortical neoplasms [23,24]. A study by Stojadinovic et al. compared 67 patients with conventional ACC and 37 patients with adrenocortical adenoma (ACA). All patients with microscopic tumor necrosis were diagnosed with ACC, and the proportion of patients with positive or unknown microscopic margins was 60.3% in ACC, whereas all patients with ACA had negative microscopic margins. Our findings are consistent with those reported by Stojadinovic et al. We found that the proportion of patients with microscopic tumor necrosis was significantly higher among those who experienced recurrence when compared with those who did not experience recurrence. Considering the status of the pathological margin, all patients with recurrence exhibited an indeterminate margin, while only 63% of patients without recurrence showed an indeterminate margin. In contrast to the aforementioned study results, no patients showed positive involvement of resection margin. This finding could be due to the fact that all patients with an indeterminate margin had specimen fragmentation during the intraoperative retrieval. Accordingly,

retrieval of the tumor as a whole during surgery is crucial to accurately assess the pathological margin. The recurrence in one patient with oncocytoma could also be associated with such indeterminate status of pathological margins.

Zhang et al. have reported Ki-67 labeling index values with 20 and 3% cutoff values as independent prognostic factors for overall survival and recurrence-free survival, respectively, in 66 patients with ACC [25]. Conversely, in the present study, the highest Ki-67 value was 5.9%, and although there was no statistical difference, the mean value in patients without recurrence was unexpectedly higher. Considering 24 patients with ACC, Angelousi et al. have reported that 66.7% of patients with recurrence exhibited p53 expression of ≥50%, while p53 expression ranged from 21−50% in 75% of patients without recurrence [13]. In the present study, the p53 expression ranged from 5 to 33% in all patients. These findings suggest that the relationship between the Ki-67 labeling index and the aggressiveness of OANUMP or oncocytoma, as well as the relationship between p53 expression and aggressiveness, may be limited.

Duregon et al. have reported that a Helsinki score >8.5 is a diagnostic factor for ACC, and a score of >17 could help predict metastasis in adrenal cortical neoplasm [18]. In addition, the authors reported a reticulin algorithm incorporating an altered reticulin framework, indicating the malignancy of adrenal cortical neoplasms [15]. In the present study, most patients with recurrence had a Helsinki score of 8.5–17, while the majority of patients without recurrence had a Helsinki score ranging between 0–8.5. In addition, most patients with recurrence exhibited malignancy on the reticulin algorithm, whereas the majority of patients without recurrence did not exhibit malignancy. Accordingly, these findings suggest that the Helsinki score >8.5 and malignancy according to the reticulin algorithm can be valuable in predicting the aggressiveness in OANUMP or oncocytoma.

Peri-adrenal soft tissue extension is one of the nine criteria proposed by Wieneke for pediatric adrenal cortical neoplasms. Based on these criteria, ≥4 points were found to be indicative of malignancy in pediatric patients [26]. Although the current study included adult patients, the proportion of patients with peri-adrenal soft tissue extension was higher among those with recurrence than among those

without recurrence (33% *vs.* 9%). These results suggest the possibility that peri-adrenal soft tissue extension might be an indicator of aggressiveness in OANUMP or oncocytoma.

The current study suggests the following six risk factors as predictors of poor prognosis in patients diagnosed with OANUMP or oncocytoma following adrenalectomy. Prognostic consideration is needed in patients presenting pathological necrosis, a Helsinki score >8.5, or having indeterminate resection margins. Additionally, if a preoperative CT scan displays three malignant features, malignancy is suspected based on the reticulin algorithm. Evidence of peri-adrenal soft tissue extension could also be indicative of disease aggressiveness. Conversely, Ki-67, p53, and β-catenin, known factors associated with the aggressiveness of adrenocortical neoplasm, were found to have a low likelihood of being risk factors. Accordingly, implementing short-term and close clinical follow-up for patients exhibiting the above-listed risk factors should be considered to ensure timely and appropriate treatment.

This study has several limitations. First, this was a retrospective single-center study, and there may be a selection bias in the study population. Second, the sample size was markedly small to demonstrate a statistically significant trend. Consequently, we identified several factors associated with recurrence through univariate analysis, although we were unable to identify any independent factors associated with poor prognosis. Third, during the adrenalectomy, all fragmented specimens were evaluated as having indeterminate resection margins. Consequently, accurate microscopic assessment of the resection margins of these specimens was unavailable. Nevertheless, to the best of our knowledge, this is the first report to identify risk factors associated with the aggressiveness of OANUMP, highlighting a substantial strength.

**Conclusions**

In patients with OANUMP or oncocytoma, the presence of pathological necrosis, Helsinki score >8.5, and indeterminate pathological resection margin after adrenalectomy could be considered risk factors to predict poor prognosis, such as recurrence. Moreover, the presence of peri-adrenal soft tissue extension, malignant features on the CT scan, and malignancy on the reticulin algorithm indicate the

possibility of future recurrence. These factors could be considered potential risk factors for predicting poor prognosis and may warrant short-term regular follow-ups for patients diagnosed as OANUMP or oncocytoma within OANs. However, to implicate them as individual risk factors conclusively, investigations among larger populations are needed.

**Reference**

[1] Xiao GQ, Pertsemlidis DS, Unger PD. Functioning adrenocortical oncocytoma: a case report and review of the literature. Ann Diagn Pathol. 2005;9(5):295-7.

[2] Wong DD, Spagnolo DV, Bisceglia M, Havlat M, McCallum D, Platten MA. Oncocytic adrenocortical neoplasms--a clinicopathologic study of 13 new cases emphasizing the importance of their recognition. Hum Pathol. 2011;42(4):489-99.

[3] Bisceglia M, Ludovico O, Di Mattia A, et al. Adrenocortical oncocytic tumors: report of 10 cases and review of the literature. Int J Surg Pathol. 2004;12(3):231-43.

[4] Mearini L, Del Sordo R, Costantini E, Nunzi E, Porena M. Adrenal oncocytic neoplasm: a systematic review. Urol Int. 2013;91(2):125-33.

[5] Fewings E, Khoo Sert Kim S, Larionov A, et al. Investigating the clinical, pathological and molecular profile of oncocytic adrenocortical neoplasms: a case series and literature review. Endocr Oncol. 2021;1(1):33-44.

[6] Ramachandran MS, Reid JA, Dolan SJ, Farling PA, Russell CF. Laparoscopic adrenalectomy versus open adrenalectomy: results from a retrospective comparative study. Ulster Med J. 2006;75(2):126-8.

[7] Eldahshan S, Celia A, Zeccolini G, Guerini A, Breda G. Laparoscopic transperitoneal adrenalectomy for adrenocortical oncocytoma. Arch Ital Urol Androl. 2008;80(2):82-4.

[8] Chin CP, Grauer R, Ucpinar B, Menon M, Si Q, Badani KK. Oncocytic adrenocortical neoplasm of borderline uncertain malignant potential diagnosed after robot-assisted adrenalectomy case report. BMC Urol. 2023;23(1):60.

[9] Kanitra JJ, Hardaway JC, Soleimani T, Koehler TJ, McLeod MK, Kavuturu S. Adrenocortical oncocytic neoplasm: A systematic review. Surgery. 2018;164(6):1351-9.

[10] Juliano JJ, Cody RL, Suh JH. Metastatic adrenocortical oncocytoma: a case report. Urol Oncol. 2008;26(2):198-201.

[11] Boland G, Blake MA, Boland G, SpringerLink. Adrenal Imaging. 1st 2009. ed. Contemporary Medical Imaging. Totowa, NJ: Humana Press : Imprint: Humana; 2009.

[12] Kebebew E. Adrenal Incidentaloma. N Engl J Med. 2021;384(16):1542-51.

[13] Angelousi A, Kyriakopoulos G, Athanasouli F, et al. The Role of Immunohistochemical Markers for the Diagnosis and Prognosis of Adrenocortical Neoplasms. J Pers Med. 2021;11(3).

[14] Fonseca D, Murthy SS, Tagore KR, et al. Diagnosis of Adrenocortical Tumors by Reticulin Algorithm. Indian J Endocrinol Metab. 2017;21(5):734-7.

[15] Duregon E, Fassina A, Volante M, et al. The reticulin algorithm for adrenocortical tumor diagnosis: a multicentric validation study on 245 unpublished cases. Am J Surg Pathol. 2013;37(9):1433-40.

[16] Volante M, Bollito E, Sperone P, et al. Clinicopathological study of a series of 92 adrenocortical carcinomas: from a proposal of simplified diagnostic algorithm to prognostic stratification. Histopathology. 2009;55(5):535-43.

[17] Pennanen M, Heiskanen I, Sane T, et al. Helsinki score-a novel model for prediction of metastases in adrenocortical carcinomas. Hum Pathol. 2015;46(3):404-10.

[18] Duregon E, Cappellesso R, Maffeis V, et al. Validation of the prognostic role of the "Helsinki Score" in 225 cases of adrenocortical carcinoma. Hum Pathol. 2017;62:1-7.

[19] Huang CP, Dai MS, Kao CC, Tsai WC, Yu CP. A Case of Oncocytic Adrenal Cortical Neoplasm with Uncertain Malignant Potential Turned Out to Be Oncocytic Adrenal Cortical Carcinoma with Distant Metastasis: Could Pathology Do Better Initially? Medicina (Kaunas). 2022;58(7).

[20] Shenouda M, Brown LG, Denning KL, Pacioles T. A Case of Oncocytic Adrenocortical Neoplasm of Borderline (Uncertain) Malignant Potential. Cureus. 2016;8(6):e638.

[21] Peppa M, Karamitopoulou E, Nikolopoulos P, et al. Large adrenal oncocytoma with uncertain malignant potential: case report and review of literature. Endocr Pract. 2010;16(4):641-5.

[22] Khan M, Caoili EM, Davenport MS, et al. CT imaging characteristics of oncocytic adrenal neoplasms (OANs): comparison with adrenocortical carcinomas. Abdom Imaging. 2014;39(1):86-91.

[23] Pearlstein SS, Conroy PC, Menut KC, et al. Evaluation of Necrosis as a Diagnostic and Prognostic Indicator in Adrenocortical Carcinoma. JAMA Surg. 2021;156(12):1173-4.

[24] Stojadinovic A, Brennan MF, Hoos A, et al. Adrenocortical adenoma and carcinoma:

histopathological and molecular comparative analysis. Mod Pathol. 2003;16(8):742-51.

[25] Zhang F, Zhang F, Liu Z, Wu K, Zhu Y, Lu Y. Prognostic Role of Ki-67 in Adrenocortical Carcinoma After Primary Resection: A Retrospective Mono-Institutional Study. Adv Ther. 2019;36(10):2756-68.

[26] Wieneke JA, Thompson LD, Heffess CS. Adrenal cortical neoplasms in the pediatric population: a clinicopathologic and immunophenotypic analysis of 83 patients. Am J Surg Pathol. 2003;27(7):867-81.

## 국문요약

호산성 부신피질 과립세포종은 진단이 어렵고 매우 희귀한 부신 종양이다. 본 연구는 부신우연종으로 부신절제술을 시행한 후 불확실 악성 잠재력의 호산성 부신피질 과립세포종 및 양성 호산성 부신피질 과립세포종으로 진단된 환자를 대상으로 불량한 예후와 관련된 위험 요인을 분석하는 것이다.

2002년 2월부터 2022년 5월까지 부신우연종으로 부신절제술을 시행한 후 불확실 악성 잠재력의 호산성 부신피질 과립세포종 및 양성 호산성 부신피질 과립세포종으로 진단된 환자 20명을 후향적으로 분석하였다. 이에 대해 병리학적 재검토를 시행하였고, 호산성 부신피질 과립세포암종으로 재 진단된 6명을 제외하고 총 14명의 환자가 연구에 포함되었다. 환자는 재발 여부에 따라 두 그룹으로 나누었고 두 그룹간 임상병리학적 및 영상학적 특징을 분석하였다. 특히, 부신 전산화단층촬영상의 특징과 부신피질악성종양과 관련된 병리학적 특징을 분석하였다.

불확실 악성 잠재력의 호산성 부신피질 과립세포종 및 양성 호산성 부신피질 과립세포종 환자 14명중 3명(21%) 에서 재발이 확인되었으며, 이중 2명(67%)은 불확실 악성 잠재력의 호산성 부신피질 과립세포종, 1명(33%)은 양성 호산성 부신피질 과립세포종 환자였다. 수술 후 병리소견에서 괴사를 보이는 환자의 비율은 재발이 없는 그룹에 비해 재발 그룹에서 더 높았다(66.7% 대 9.1%, p=0.031). 재발 그룹에서 헬싱키점수가 8.5를 초과하는 환자의 비율이 재발이 없는 그룹에 비해 더 높았다 (66.7% 대 9.1%, p=0.031). 비결정 경계를 보이는 환자의 비율이 재발이 없는 그룹에 비해 재발 그룹에서 더 높았다 (100% 대 63.6%, p=0.051). 부신 전산화 단층촬영 검사상 악성 특징을 보이는 환자의 비율, 병리소견에서 부신 주위 연부조직으로의 침범이 보이는 환자의 비율, 레티큘린 알고리즘 상 악성을 보이는 환자의 비율이 재발이 없는 그룹에 비해 재발 그룹에서 더 높았으나, 통계적으로 유의한 차이는 없었다. Ki-67, p53 의 발현 정도, β-catenin 의 양성 여부는 재발이 있는 환자와 없는 환자간 유의한 차이는 없었다.

결론적으로, 불확실 악성 잠재력의 호산성 부신피질 과립세포종 및 양성 호산성 부신피질 과립세포종 환자에서 수술 후 병리소견상 괴사, 헬싱키점수 8.5 초과, 비결정 병리학적 절제연이 단변량 분석에서 재발과 높은 연관성이 있었다. 부신 전산화 단층촬영 검사상 악성 특징, 병리소견상 부신 주위 연부조직으로의 침범, 레티큘린 알고리즘 상 악성을 보이는 것은 재발 가능성과 관련이 있었다. 이러한 요인들은 불량한 예후의 위험요인일 수 있으며, 이러한 소견을 보이는 환자들은 정기적인 단기 추적관찰이 필요할 수 있다.