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Clinical, pathologic, and imaging characteristics of pituitary null cell adenomas as defined according to the 2017 World Health Organization criteria: a case series from two pituitary centers

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Abstract

Purpose The 2017 World Health Organization classification of pituitary tumors redefined pituitary null cell adenomas (NCAs) by restricting this diagnostic category to pituitary tumors that are negative for pituitary transcription factors and adenohypophyseal hormones. The clinical behavior of this redefined entity has not been widely studied, and this is a major shortcoming of the classification. This study evaluated the imaging and clinical features of NCAs from two pituitary centers and compared them with those of gonadotroph adenomas (GAs).

Methods Imaging, pathologic, and clinical characteristics of NCAs and GAs were retrospectively reviewed. Tumor immunohistochemistry was performed to confirm absence of adenohypophyseal hormones and pituitary transcription factor expression.

Results Thirty-one NCAs were compared with 38 GAs. NCAs were more likely to invade the cavernous sinus (15/31 [48%] vs. 5/38 [13%], $P = .003$) and had a higher proliferative index (i.e., MIB-1 > 3%, 11/31 [35%] vs. 5/38 [13%], $P = .04$). Gross total resection was less likely in the NCA group (19/31 [61%] vs. 33/38 [87], $P = .02$). Progression-free survival was worse in the NCA cohort (5-year progression-free survival, 0.70 vs. 1.00; $P = .011$, by log-rank test).

Conclusions Compared with GAs, NCAs are more invasive at the time of presentation and have a more aggressive clinical course. This study provides evidence that NCAs represent a distinct clinicopathologic entity with behavior that differs adversely from that of GAs. This may inform clinical decision-making, including frequency of postoperative tumor surveillance and timing of adjunctive treatments.

Keywords Gonadotroph adenoma · Gonadotroph tumors · Null cell adenoma · Null cell pituitary tumor pituitary

Abbreviations

GA	Gonadotroph adenoma
NCA	Null cell pituitary adenoma
WHO	World Health Organization

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T-Pit T-box family member that is the product of TBX19
SF-1 Steroidogenic factor 1
PIT-1 Pituitary-specific POU class homeodomain transcription factor 1

Introduction

Null cell pituitary adenomas (NCAs) are clinically nonfunctioning pituitary lesions that either present with symptoms of mass effect, such as hypopituitarism or chiasmopathy, or are discovered incidentally [1]. The term NCA was first introduced in 1980 by Kovacs et al. [2] to describe a clinically nonfunctioning pituitary adenoma that also lacked clinical or morphologic markers indicating a cellular lineage. According to the World Health Organization (WHO) 2004 guidelines [3], this entity was defined as a pituitary tumor that lacked immunoreactivity for anterior pituitary hormones on pathologic staining. Application of these criteria has since proved problematic, in part because of the challenge of distinguishing between clinically hormone-negative pituitary tumors, especially gonadotroph adenomas (GAs) and NCAs. It has been shown that a significant proportion of GAs are immunohistochemically negative for gonadotropins. In addition, other tumors, including corticotroph tumors, can also be immunohistochemically negative for the hormone [4–6].

In 2017, the WHO published updated pathology guidelines for endocrine tumors [7]. One of the major shortcomings of the update is the absence of robust prognostic information. The two notable changes in these guidelines relevant for this article include the classification of adenohypophyseal tumors into cell lineages on the basis of expression of key transcription factors and the redefinition of the NCA entity in light of previous evidence [5, 6, 8]. The transcription factors responsible for cytodifferentiation included in the guidelines have been shown to be responsible for corticotroph differentiation (i.e., T-box family member that is the product of TBX19 [Tpit, T-Pit), gonadotroph differentiation (i.e., steroidogenic factor 1 [SF-1]), and mammosomatotroph differentiation (i.e., pituitary-specific POU class homeodomain transcription factor 1, PIT-1) [5, 9, 10]. The definition of NCA was refined to include not only the absence of adenohypophyseal hormone expression but also the absence of immunoreactivity for those transcription factors important in pituitary cell lineage differentiation [6, 8]. With use of these updated and more precise criteria, fewer tumors are now diagnosed as NCAs, because many tumors previously diagnosed as NCAs likely represented hormone-negative GAs [5, 8]. The clinical behavior and imaging characteristics of this newly redefined NCA have not been well characterized. The aim of this study is to describe the imaging, pathologic, and clinical characteristics of NCAs and compare them with those of GAs to inform medical decision-making, follow-up, and patient counseling.

Methods

The study adhered to the principles in the *US Code of Federal Regulations*, Title 45, Part 46, "Protection of Human Subjects" (revised January 15, 2009). The study was approved by the St. Joseph's Hospital Institutional Review Board (Phoenix, AZ) and the Research Ethics Board of the University Health Network (Toronto, ON, Canada). Patient informed consent was waived by the ethics boards at each site. STROBE guidelines for cohort studies were used as the reporting guidelines (http://www.strobe-statement.org).

Patient population

Pathology databases from the Barrow Neurological Institute (Phoenix, AZ) and University Health Network (Toronto, ON, Canada) were queried for patients with a histopathologic diagnosis of NCA following transsphenoidal surgery between 2007 and 2017. A subset of these patients were included in an earlier report [6]. Patient demographic characteristics, imaging findings, Knosp grade, and treatment details were abstracted from the medical record by three of the authors (C.S., A.S.L., and J.P.A.) [11]. Tumor progression was detected by routine interval postoperative pituitary protocol MRI according to the institutional protocol.

Pathologic analysis and immunohistochemistry

The immunohistochemistry panel at both centers was performed on formalin-fixed, paraffin-embedded tissues and included testing for adenohypophyseal hormones (i.e., adrenocorticotropin, growth hormone, prolactin, α-subunit, β-thyrotropin, β-folliculotropin [β-follicle-stimulating hormone], and β-luteotropin [β-luteinizing hormone]). Pituitary transcription factors assessed included Pit1, SF-1, and Tpit. Supplementary stains included Ki-67 or MIB-1 antibody to evaluate proliferative activity by counting at least 1000 tumor cells from hot spots using manual count and/or automated image analysis nuclear algorithm (Leica Biosystems), reticulin histochemistry to assess cell architecture, cytokeratin 20, low molecular weight cytokeratin (CAM 5.2), synaptophysin, p27, FGFR4, estrogen receptor alpha, and E-cadherin. All slides were reviewed and scored by a board-certified neuropathologist or Royal College—recognized endocrine pathologist who was also involved in the most recent WHO classification of pituitary endocrine tumors. Tissue samples stained for Tpit, SF-1, and Pit-1 were scored as positive or negative. No equivocal cases were identified. Immunohistochemistry methodology details are included

in Supplementary Tables 1 and 2. At Barrow Neurological Institute, pituitary transcription factors were analyzed beginning in 2017. All specimens that were classified as NCAs before the implementation of immunohistochemistry for transcription factors were subsequently analyzed for transcription factors for inclusion in this study. At University Health Network, transcription factors were assessed during the entire survey period of this study. The control group for the study consisted of patients initially characterized as having NCAs during the survey period who, on subsequent testing for transcription factors, were noted to have GAs due to SF-1 expression.

Statistical analysis

Student's *t* tests were used to compare the mean difference between the two groups. A Kaplan–Meier analysis was used to estimate progression-free survival over time. *P* values < .05 were considered statistically significant. STATA version 14.2 (StataCorp, LLC, College Station, TX) and NCSS version 10 (NCSS, LLC, Kaysville, UT) were used for statistical analyses.

Results

Thirty-one cases of pathologically confirmed NCA based on WHO 2017 criteria were identified from 2007 to 2017 at two pituitary centers. A control group of 38 cases of GA from the same time period was identified. Patients in the control group had previously received a diagnosis of NCA on the basis of the WHO 2004 criteria (diagnosed at Barrow Neurological Institute) and the tumors were reclassified as GA because of the presence of SF-1 expression on retesting for this study. The mean (standard deviation) cohort follow-up period was 5.0 (5.2) years. Age at presentation and tumor size were similar between groups ($P \geq .30$) (Table 1). There were more women in the NCA group, but the difference did not reach statistical significance (18/31 [58.1%] vs. 13/38 [34.2%], $P = .06$). NCAs were more likely to invade the cavernous sinus (15/31 [48.4%] vs. 5/38 [13.2%], $P = .003$) and have a high proliferative index (i.e., MIB-1 or Ki67 > 3%; 11/31 [35.5%] vs. 5/38 [13.2%], $P = .04$). Patients with NCA were less likely to present with headache than were patients with GA (5/31 [16.1%] vs. 25/38 [65.8%], $P < .001$]. Apoplexy and visual disturbance were similar between groups ($P \geq .46$). Gross total resection was less likely in the NCA group (19/31 [61.3%] vs. 33/38 [86.8], $P = .02$).

The proportion of patients with tumor progression-free survival was lower in the NCA cohort at the mean cohort follow-up of 5 years (5-year progression-free survival, 0.70 vs. 1.00; $P = .011$, by log-rank test) (Fig. 1). Five patients with NCAs (16.1%) and 2 patients with GAs (5.3%) underwent radiotherapy for recurrent or residual tumor ($P = .23$). Four patients with NCAs (12.9%) and 3 patients with GAs (7.9%) underwent additional tumor surgery ($P = .69$).

Discussion

The aim of this study was to compare the newly redefined NCA with GA with respect to clinical behavior and imaging presentation to help guide clinical practice. The key findings of the study are that, compared with GAs, NCAs were more prevalent among women, more invasive at the time of presentation (i.e., more likely to involve cavernous sinus invasion), had a higher proliferative index, and were associated with a more aggressive clinical course, indicated by a shorter progression-free survival. This study provides additional evidence that true NCAs represent a distinct clinicopathologic entity with a behavior that differs adversely from that of GAs. Our findings challenge previously held orthodoxy that GAs and NCAs exhibit similar behavior. Because of their behavior, NCAs may require more diligent postoperative surveillance. Health care practitioners may also weigh the benefits of adjuvant treatment (e.g., radiosurgery) in patients with NCAs who have residual disease after surgery, given that these lesions appear more likely to recur than GAs.

NCAs are thought to arise from a pluripotent precursor cell that can differentiate into different cell lineages. The transcription factors included in the WHO 2017 classification of pituitary tumors are thought to be lineage-determining factors resulting in the terminal differentiation of pituitary cells. Recently, there have been considerable gains in our understanding of pituitary cell origins and plasticity [1, 12, 13]. Vankelecom and colleagues, in an excellent review article, indicated that "multiple signaling molecules and transcriptional activators or repressors are required during the early phases of pouch commitment, patterning, expansion, and lineage determination and are expressed in spatial and temporal patterns, partly distinct, partly overlapping" [14]. We and others have speculated that the less differentiated nature of the tumor explains its more aggressive clinical course [6, 15]. Our results extend prior work. Some of us have previously investigated NCAs [6]. In that study, Balogun and colleagues discovered that NCAs have a higher proliferative index and shorter tumor doubling time than GAs. As in the current study, the tumors were more common among women and occurred in the sixth decade of life. Our data also corroborate Batista et al. [16], who reported a female preponderance among patients with NCAs and a male preponderance among patients with GAs, although the reason for this sex difference is unclear. Importantly, our study differs from earlier work by applying the WHO 2017 pathologic criteria, rather than 2004 criteria, and by

Table 1 Comparison of clinical, pathologic, and imaging features of patients with null cell adenomas (NCAs) and patients with gonadotroph adenomas (GAs)

Characteristic	NCA group ($n=31$)	GA group ($n=38$)	P value
Age, mean (SD), years	53.6 (14.2)	56.7 (17.2)	.50
Sex			.06
Male	13 (41.9)	25 (65.8)	
Female	18 (58.1)	13 (34.2)	
Maximum adenoma diameter, mean (SD), cm^3	2.7 (0.9)	2.5 (1.2)	.30
CS invasion[a]	15 (48.4)	5 (13.2)	**.003**
MIB-1 or Ki67			**.04**
> 3%	11 (35.5)	5 (13.2)	
≤ 3%	20 (64.5)	33 (86.8)	
Suprasellar extension	28 (90.3)	34 (89.5)	> .99
Extent of adenoma resection			
Gross total	19 (61.3)	33 (86.8)33	**.02**
Subtotal	12 (38.7)	5 (13.2)5	
Subsequent treatment			
Resection	4 (12.9)	3 (7.9)	.69
Radiation	5 (16.1)	2 (5.3)	.23
Presenting symptom[b]			
Vision disturbance	21 (67.7)	22 (57.9)	.46
Headache	5 (16.1)	25 (65.8)	**< .001**
Diplopia	0 (0)	4 (10.5)	.12
Hypopituitarism of at least one hormone axis[c]	1 (3.7)	7 (20.5)	.056
Hypothyroidism	1 (3.7)	5 (14.7)	
Adrenal insufficiency	1 (3 .7)	3 (8.8)	
Hypogonadism	0	2 (5.3)	
Diabetes insipidus	0	0	
Apoplexy	4 (12.9)	4 (10.5)	> .99
Incidental	3 (9.7)	0 (0)	.09

Data are no. (%) of patients unless otherwise indicated. Boldface type indicates statistical significance

CS cavernous sinus, *SD* standard deviation

[a]Knosp grade 3 or 4

[b]Patients may present with more than one symptom

[c]Excludes patients with apoplexy

the addition of patient data from a second pituitary center to the data set.

We chose to compare NCAs with GAs for two reasons. First, previous work has suggested that NCAs and GAs were tumor types with similar clinical behavior and that the pathologic distinction had no clinical relevance. However, by applying the new pathologic definition, it is likely that many tumors that were previously determined to be NCAs were actually hormone-negative pituitary endocrine tumors, and especially hormone-negative GAs, and our previous clinical understanding of NCAs was influenced heavily by the clinical features of GAs [4, 17–20]. Second, we wanted to provide clinical context for the behavior of NCAs using a comparator that clinicians have experience managing.

The study has limitations worthy of further discussion. The first limitation is that, despite combining results from two dedicated pituitary institutions, there are a relatively small number of NCA cases available for analysis. This is due to the rarity of these tumors. A retrospective review of 1055 pituitary tumors from 1169 transsphenoidal pituitary tumor resections from the pathology files of University Health Network reported that NCAs accounted for 4.5% of all pituitary tumors when using adenohypophysial pituitary transcription factors along with pituitary hormones and other biomarkers [4]. In a select series, Nishioka et al. [5] reported that NCAs likely represent less than 1% of pituitary tumors. The second limitation is that, because our series consists of 31 cases, it does not have the statistical power to investigate independent predictors of tumor recurrence. For example, we cannot perform a multivariate analysis to determine whether NCAs are more likely to recur because they are more invasive (e.g., associated with cavernous sinus invasion) at the time of presentation or because of other biological factors, such as higher proliferative index. In addition, we

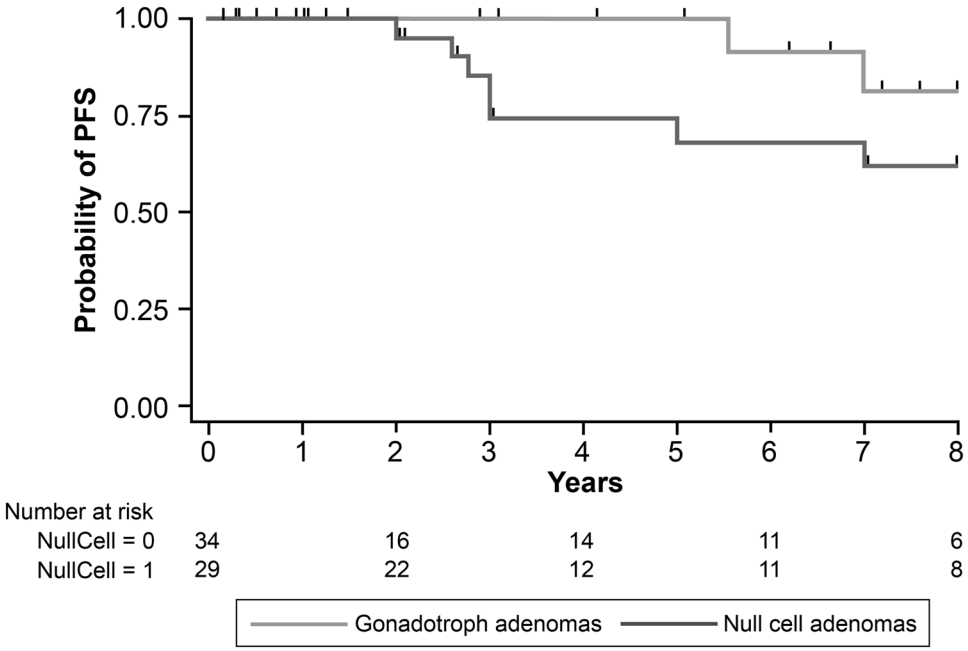

Fig. 1 Kaplan–Meier survival analysis demonstrating progression-free survival (PFS) for patients with null cell adenomas and patients with gonadotroph adenomas ($P = .011$ at 5 years). Used with permission from Barrow Neurological Institute, Phoenix, Arizona

observed a higher incidence of hypopituitarism in patients with GAs ($P = .056$), but it is not clear to us why this would be so given that the groups had tumors of similar size and had similar rates of suprasellar extension. Additional larger multicenter series are needed to determine if this observation holds and is an important future direction. Finally, this is a retrospective clinical study with mean follow-up of slightly longer than 5 years. Given that pituitary adenomas are benign lesions, longer follow-up would illuminate whether the differences between NCAs and GAs with respect to clinical characteristics persist beyond 5 years.

Conclusions

When compared with GAs, NCAs occur more commonly among women, are more invasive at the time of presentation, and are associated with a more aggressive clinical course and shorter progression-free survival. This study provides evidence that NCAs represent a distinct clinicopathologic entity with a behavior that differs adversely from that of GAs. This information may impact clinical decision-making, including the frequency of postoperative tumor surveillance and timing of adjunctive treatments.

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Compliance with ethical standards

Conflict of interest Dr. Little is a stockholder in Kogent Surgical, LLC, and has stock options in SPIWay, LLC. Dr. Yuen is a consultant for Pfizer, Novo Nordisk, Sandoz, Aeterna Zentaris, Novartis, Corcept Therapeutics, and Strongbridge. Drs. White, Mooney, Felicella, Zadeh, Almeida, Gentili, Mete, Bernat, Eschbacher, and Mr. Stephens have no disclosures.

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