The 10 Hounsfield units unenhanced computed tomography attenuation threshold does not apply to cortisol secreting adrenocortical adenomas

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Abstract

Context: Computed tomography (CT) unenhanced attenuation value of <10 Hounsfield units (HU) has an excellent specificity (98%) to diagnose lipid-rich adrenocortical adenomas (ACAs) with a weaker sensitivity (71%).
Objective: To determine from a routine clinical perspective if unenhanced attenuation value is influenced by cortisol secretion in ACAs.
Design: This was a retrospective study of cases collected between 2009 and 2012.
Setting: This study was conducted in a tertiary-care university hospital.
Patients: Seventy-two patients operated on for an ACA (Weiss score ≤ 2) were analysed. Thirty-four patients had an ACA oversecreting cortisol (Cush-ACA). Thirty-eight patients had an ACA without cortisol oversecretion (Non Hyper-ACA).
Main outcome measure: CT unenhanced attenuation value was correlated with the functional status. The Weiss score items were analysed.
Results: Among the 34 patients with a Cush-ACA a minority ($n=7$) had an unenhanced attenuation value under 10 HU. Among the high precontrast density (>10 HU) Cush-ACAs, washout analysis after contrast administration was consistent with the benign nature of the tumor in $\sim$60% of the cases. Less than 25% clear cells (lipid-rich cells), a Weiss score item, was present in 50% of the Cush-ACAs in favour of a lipid-poor content.
Conclusions: Unenhanced attenuation value has a poor sensitivity to diagnose an ACA in case of cortisol oversecretion due to poor lipid content. Nevertheless, the accuracy of washout analysis was preserved in the group of Cush-ACAs.

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Introduction

Adrenocortical adenoma (ACA) may be discovered using two diagnostic modalities: as an incidental radiologic finding or as part of the investigation of steroid excess. Visualization of an adrenal incidentaloma on cross-sectional imaging is a frequent situation, with an estimated frequency around 4% in the middle age (1, 2, 3). Different histological types may be encountered, but non-functioning ACAs represent more than 70% of all incidentalomas (4). Pheochromocytomas, adrenocortical carcinomas and adrenal metastases are less common disorders but need to be considered because of their serious consequences for the patient. Cortisol-secreting ACA is rarely discovered as an adrenal incidentaloma, but rather usually in the workup of a Cushing's syndrome (5).

Although the final diagnosis of an ACA relies on the Weiss score after histological examination (6, 7), some specific radiological characteristics of ACA are highly suggestive of a benign lesion (8). An ACA is suggested on a computed tomography (CT) by the presence of a regular border, a maximum size measurement <4 cm, homogeneity of the lesion and an unenhanced density of <10 Hounsfield units (HU). Pre-contrast attenuation value of <10 HU on CT is suggestive of a lipid-rich tumor composed of benign steroidogenic cells. Analysis of the literature on CT reveals that a precontrast density below 10 HU has 98% specificity to diagnose an ACA, with a weaker sensitivity of 71% (9). Approximately 30% of ACAs are lipid-poor, resulting in a higher pre-contrast attenuation value, which accounts for the lower sensitivity. Nonetheless, many of these lipid-poor ACAs will be identified by rapid washout after contrast administration. This is in contrast to malignant tumors, which have poor contrast washout as a result of capillary leakage (10).

To our knowledge it has never been systematically investigated in clinical practice whether the functional status might modify the lipid content of an adenoma, and therefore the pre-contrast CT attenuation value. In Cush-ACAs the hyperfunctional status may decrease the intracytoplasmic lipid droplets containing ester of cholesterol necessary for cortisol synthesis. The present study aims to answer this question, especially given the clinical importance put on the radiological findings.

Subjects and methods

Patients

This was a retrospective study of 72 patients who underwent resection of an ACA between 2009 and 2012 in Cochin Hospital, Paris. Patients were included in the analysis if the following data were available: unenhanced CT attenuation value of the adrenal lesion, pre-operative 24-h urinary free cortisol (UFC) and a Weiss score on the histopathology report of the resected ACA.

Patients with an adrenal incidentaloma were investigated according to established guidelines (1, 11) and patients with Cushing's syndrome were investigated as recommended by the Endocrine Society (12). Hormonal investigations were performed as previously reported (13, 14). The tests commonly used were 24-h UFC, basal plasma adrenocorticotrophin (ACTH), late-night serum and/or salivary cortisol and 1 mg dexamethasone suppression test. Inadequate cortisol suppression after 1mg overnight dexamethasone test was defined by the cutoff value of 1.8 μg/dl (50 nmol/l) (12). Based on clinical and biochemical parameters, patients are usually classified as having non-secreting adenoma, subclinical hypercortisolism and Cushing's syndrome. As our objective was to determine if a correlation may exist between the CT-unenhanced attenuation value and the degree of cortisol hypersecretion, the 72 patients were arbitrarily divided into two groups based on 24-h UFC. Those with evidence of cortisol excess with elevated UFC were classified as the Cush-ACA group whilst those with normal UFC, with or without subtle cortisol oversecretion, classified as the Non Hyper-ACA group. In this latter group, 79% of patients had an abnormal response to dexamethasone. In the Non Hyper-ACA group, 36 adenomas were discovered incidentally and two were found in the workup of a clinical Cushing's syndrome. However, in the Cush-ACA group, four were incidentaloma and 30 were found in the assessment of a clinical Cushing's syndrome. In the Non Hyper-ACAs group, the cause of the surgical penalty was the initial size for four patients (10.5%), a growing tumor for five patients (13.2%), a radiologically indeterminated lesion for nine patients (23.7%) and a subclinical Cushing syndrome for 20 patients (52.6%).

Image analysis

Features analysed on routine CT scanning included the size, shape and unenhanced attenuation value of the ACA. All the ACAs were homogenous tumors with a regular border. Homogeneity of the tumor was determined by the radiologist who characterized the adrenal lesion. Attenuation evaluation was based on a region of interest located at midpoint of the lesion, covering two-thirds of the mass. Unenhanced attenuation value was expressed in Hounsfield

units which represent a normalized index of X-ray attenuation, in relation with the composition of the tissue imaged. Where available, the rapidity of i.v. contrast washout was recorded. The absolute contrast washout (APW) was calculated by the formula: APW = (enhanced − delayed)/(enhanced − unenhanced) × 100. An APW of 60% or more is in favour of an ACA. The relative contrast washout (RPW) was calculated with the formula: RPW = (enhanced − delayed)/enhanced × 100. A relative washout of 40% or more was in favour of an ACA. All the patients provided written informed consent.

Weiss score analysis

The pathological report allowed us to select exclusively benign adrenocortical tumors defined by a Weiss score ≤2. The nine Weiss criteria were recorded by two pathologists specialized in adrenal pathology (F T and M S). The diagnostic performance of the Weiss score system is high among expert pathologists. Borderline cases with one or two Weiss score criteria can be observed, but a secondary malignant behaviour is extremely rare. One of the nine Weiss score criteria evaluates tumor lipid content by quantifying the percentage of clear or vacuolated cells. These cells correspond to spongiocytes which are rich in lipid droplets, in contrast with lipid-poor compact cells. The presence of less than or equal to 25% of clear cells equates to one point in the Weiss scoring system, which suggests that the tumor is lipid-poor.

Statistics

Data are reported using median and range ± S.D., or percentages as appropriate. Comparison of quantitative variables was done using *t*-test. Given the small numbers of subjects in some groups of interest, we used non-parametric statistical methods to study the relationship between qualitative variables when appropriate (Fisher's exact χ^2 test). We used two-tailed tests, and P values <0.05 were considered significant. Statistical analysis was performed using SAS version 9.2 (SAS institute, Inc., Cary, NC, USA).

Results

Patient characteristics

The demographic, hormonal and radiological data of the 38 patients from the Non Hyper-ACA group are presented in Supplementary Table 1 (see section on supplementary data given at the end of this article). Similar data are presented for the 34 patients from the Cush-ACA group in Supplementary Table 2.

These data are summarized in Table 1. The majority of the patients were female, with a male-to-female ratio of 0.29 in the Non Hyper-ACA group and 0.13 in the Cush-ACA group. This difference did not reach significance.

The median age at diagnosis was 58.6 ± 12.2 years (range 33–87) in the Non Hyper-ACA group and was significantly younger, 41.6 ± 11.7 years (range 14–66), in the Cush-ACA group ($P<0.0001$).

As expected by the definition of the two groups, cortisol secretion, expressed as 24-h UFC times upper limit of normal (× ULN), was higher in the Cush-ACA group, compared to the Non Hyper-ACA group (3.62 ± 2.95 (min 1.03–max 12.70) vs 0.44 ± 0.25 (min 0.08–max 0.99), $P<0.0001$).

Median tumor size was higher in Non Hyper-ACA group vs Cush-ACA group, though this does not reach significance (37.9 ± 8.5 mm (range 22–58) vs 33 ± 12.7 mm (range 20–65), $P=0.06$).

Unenhanced CT attenuation values

Cush-ACAs had significantly higher attenuation values at unenhanced CT than did Non Hyper-ACAs (median 23.6 ± 11.8 HU (range 0 to 41) vs 14.2 ± 15.1 HU (range –11 to 41), $P=0.004$) (Table 1).

The relationship between functional status and unenhanced CT attenuation value is illustrated in Fig. 1. Seventy-nine percent of cortisol-secreting adenomas had an unenhanced attenuation value >10 HU, and 64.7% had an unenhanced attenuation value of ≥20 HU, in

Table 1 Demographic and radiological data.

Clinical and radiological characteristics	Non Hyper ACAs ($n=38$)	Cush ACAs ($n=34$)	*P* value
Age at diagnostic (years)	58.6 ± 12.2	41.6 ± 11.7	<0.0001
Female	29 (76.3%)	30 (88.2%)	0.2
Size (mm)	37.9 ± 8.5	33 ± 12.7	0.06
Urinary free cortisol (× upper limit normal)	0.44 ± 0.25	3.62 ± 2.95	<0.0001
Unenhanced CT attenuation (HU)	14.2 ± 15.1	23.6 ± 11.8	0.004
ACAs with unenhanced CT >10 HU	21 (55%)	27 (79.4%)	0.04
ACAs (>10 HU) with washout study available	18/21	25/27	
Absolute washout ≥60%	7/18 (39%)	15/25 (60%)	0.2
Relative washout ≥40%	6/18 (33%)	16/25 (64%)	0.07

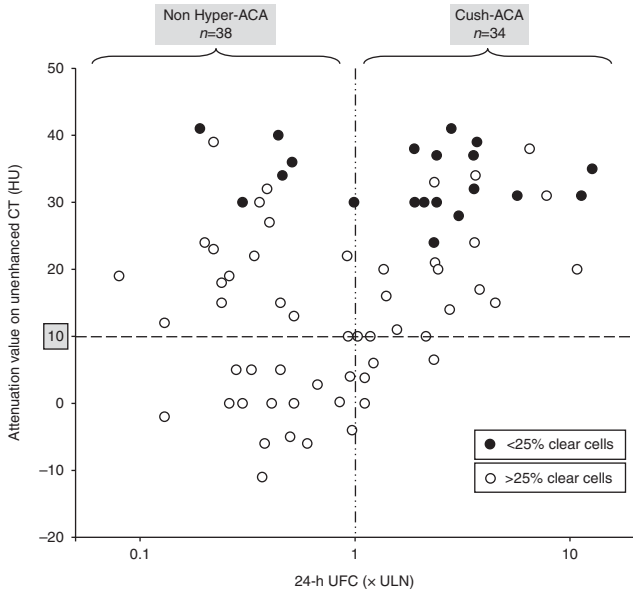

Figure 1

Relationship between cortisol secretion and unenhanced CT attenuation value. The *x*-axis represents the level of diurnal cortisol secretion expressed as 24-h urine free cortisol (UFC) times upper limit of normal (×ULN). Note that the *x*-axis is represented as a logarithmic scale. The vertical line separates the Non Hyper-ACA group (ULN ≤1) from the Cush-ACA group (ULN >1). The *y*-axis represents the unenhanced CT attenuation value expressed in Hounsfield unit (HU). The horizontal line indicates the 10 HU threshold value used in clinical practice to identify lipid-rich adenomas (below 10 HU). The adenomas with >25% of clear cells (empty circles) are compared to the adenomas with <25% of clear cells (full circles).

keeping with lipid-poor adenoma. It is worth noting that among the seven lipid-rich Cush-ACAs (unenhanced CT attenuation value ≤10 HU), five were the least over-secreting (ULN <1.25) (Supplementary Table 2).

In the Non Hyper-ACA group both lipid-rich (42%) and lipid-poor (58%) adenomas were observed, reflecting the fact that a proportion of these patients were operated on for a radiologically indeterminate lesion (i.e. high unenhanced CT attenuation value with slow washout).

A positive linear relationship was observed between the cortisol secretion and unenhanced attenuation values in the entire cohort of ACAs with a correlation coefficient equal to 0.36 ($P=0.002$) and in the Cush-ACAs group with a correlation coefficient equal to 0.38 ($P=0.027$), but not in the Non Hyper-ACAs group ($P=0.266$). In other words, for the entire cohort, a doubling of the cortisol secretion was associated with a 4 HU increase in the unenhanced attenuation value.

Contrast washout characteristics

Since use of unenhanced CT attenuation value had limitations in identifying the benign nature of cortisol-secreting adenoma, we analysed if the washout contrast study continued to perform well. Fourteen patients (37%) in the Non-Hyper ACAs group and ten patients (29%) in the Cush-ACAs group had a radiologically indeterminated mass (high unenhanced CT attenuation value with slow absolute washout).

Of the 34 Cush-ACAs, 27 had an unenhanced CT attenuation value >10 HU. A contrast washout study was available in 25 of these 27 lipid-poor adenomas. Of these 25 Cush-ACAs, the absolute percentage washout was >60% in 15 out of 25 cases (60%), in favour of an adenoma. The relative percentage washout was >40% in 16 out of 25 cases (64%), again, in keeping with an adenoma (Table 1).

Of the 38 Non Hyper-ACAs, 21 had an unenhanced CT attenuation value >10 HU. A contrast washout study was available in 18 of these 21 lipid-poor adenomas. Given that the group contained more radiologically indeterminate lesions, the percentage of tumors displaying washout characteristics in keeping with an adenoma was lower: the absolute percentage washout was in favour of an adenoma in seven out of 18 cases (39%), and the relative percentage washout was in favour of the benignity in six out of 18 cases (33%) (Table 1).

Analysis of the Weiss score criteria

Table 2 summarises the Weiss score criteria identified in the Cush-ACA group. Among the 27 lipid-poor Cush-ACA with an unenhanced CT attenuation value of >10 HU, 50% were noted to have ≤25% of clear cells. As expected, this Weiss score criterion was absent in all of the seven lipid-rich Cush-ACAs (unenhanced CT attenuation value ≤10 HU).

The only other Weiss score criterion noted in the Cush-ACA group was the finding of nuclear grade III or IV, based on Fuhrman criteria (15). This was present in 19 of the 34 (55.9%) Cush-ACAs, with ten Cush-ACA's having both high nuclear grade and low percentage of clear cells (Weiss score of 2).

Table 3 summarises the Weiss score criteria identified in the Non Hyper-ACA group. Only six of the 38 (15.8%) Non Hyper-ACAs had ≤25% clear cells.

Four other Weiss score items were recorded, but the most common was a nuclear grade score of 1, seen in

Table 2 Weiss score criteria in the Cush-ACA group.

Tumors	Weiss score (total)	Weiss score criteria								
		Nucleus			Architecture			Invasion		
		NG	MT	AM	CC	DA	NC	VI	SI	CI
Cush-ACA1	0									
Cush-ACA2	0									
Cush-ACA3	0									
Cush-ACA4	0									
Cush-ACA5	1	+								
Cush-ACA6	0									
Cush-ACA7	0									
Cush-ACA8	1				+					
Cush-ACA9	2	+			+					
Cush-ACA10	2	+			+					
Cush-ACA11	0									
Cush-ACA12	2	+			+					
Cush-ACA13	0									
Cush-ACA14	1	+								
Cush-ACA15	0									
Cush-ACA16	1				+					
Cush-ACA17	2	+			+					
Cush-ACA18	1	+								
Cush-ACA19	1	+								
Cush-ACA20	2	+			+					
Cush-ACA21	2	+			+					
Cush-ACA22	2	+			+					
Cush-ACA23	2	+			+					
Cush-ACA24	1	+								
Cush-ACA25	1	+								
Cush-ACA26	1				+					
Cush-ACA27	0									
Cush-ACA28	1	+								
Cush-ACA29	2	+			+					
Cush-ACA30	0									
Cush-ACA31	1	+								
Cush-ACA32	1	+								
Cush-ACA33	1				+					
Cush-ACA34	2	+			+					

NG, Nuclear grade; MT, mitosis; AM, atypical mitosis; CC, <25% clear cells; DA, diffuse architecture; NC, necrosis; VI, venous invasion; SI, sinusoidal invasion; CI, capsular invasion.

ten of the 38 (26.3%) Non Hyper-ACAs. This group is characterised by low Weiss score, with 68.4% having a Weiss score of zero. The Weiss score distribution is statistically different between the two groups ($P=0.004$).

In both groups, ≤25% of clear cells were only observed for ACAs with an unenhanced attenuation value above 20 HU. In the Cush-ACAs group, 70% of the adenomas with more than 20 HU attenuation value had this Weiss score criteria. In the Non Hyper-ACAs group, it was only observed in 42.9% of the adenomas (Fig. 1). This difference was not statistically significant ($P=0.162$, Fisher test), suggesting that a high unenhanced attenuation value is also associated with a small number of clear cells in non-cortisol-secreting ACAs.

Discussion

In clinical practice, an unenhanced CT attenuation value of <10 HU is widely used to distinguish lipid-rich ACAs from other adrenal tumors due to its excellent specificity (9). This threshold is a less sensitive marker, as ~30% of adenomas are lipid-poor.

Different mechanisms have been proposed as to why cholesterol esters are not stored in intracytoplasmic lipid droplets in lipid-poor ACAs. A number of studies have suggested that it is due to cortisol hypersecretion (16, 17, 18), but this hypothesis has not been explored in clinical practice.

In our retrospective analysis of a large series of ACAs, we have correlated the level of cortisol secretion with

Table 3 Weiss score criteria in the Non Hyper-ACA group.

Tumors	Weiss score (total)	Weiss score criteria								
		Nucleus			Architecture			Invasion		
		NG	MT	AM	CC	DA	NC	VI	SI	CI
Non Hyper-ACA1	0									
Non Hyper-ACA2	1	+								
Non Hyper-ACA3	0									
Non Hyper-ACA4	2	+			+					
Non Hyper-ACA5	0									
Non Hyper-ACA6	0									
Non Hyper-ACA7	0									
Non Hyper-ACA8	0									
Non Hyper-ACA9	2	+				+				
Non Hyper-ACA10	0									
Non Hyper-ACA11	0									
Non Hyper-ACA12	0									
Non Hyper-ACA13	0									
Non Hyper-ACA14	2	+			+					
Non Hyper-ACA15	0									
Non Hyper-ACA16	0									
Non Hyper-ACA17	0									
Non Hyper-ACA18	0									
Non Hyper-ACA19	0									
Non Hyper-ACA20	1	+								
Non Hyper-ACA21	0									
Non Hyper-ACA22	1	+								
Non Hyper-ACA23	2	+			+					
Non Hyper-ACA24	2						+			+
Non Hyper-ACA25	0									
Non Hyper-ACA26	2	+			+					
Non Hyper-ACA27	0									
Non Hyper-ACA28	1				+					
Non Hyper-ACA29	0									
Non Hyper-ACA30	0									
Non Hyper-ACA31	0									
Non Hyper-ACA32	0									
Non Hyper-ACA33	2	+					+			
Non Hyper-ACA34	0									
Non Hyper-ACA35	0									
Non Hyper-ACA36	0									
Non Hyper-ACA37	0									
Non Hyper-ACA38	2	+			+					

NG, Nuclear grade; MT, mitosis; AM, atypical mitosis; CC, <25% clear cells; DA, diffuse architecture; NC, necrosis; VI, venous invasion; SI, sinusoidal invasion; CI, capsular invasion.

unenhanced CT attenuation value, and we clearly demonstrate that cortisol hypersecretion is associated with high unenhanced CT attenuation values. Given that 79% of Cush-ACAs had an unenhanced CT attenuation value of >10 HU, this radiological threshold lacks sensitivity in this type of ACA. However, in our series, the contrast washout studies identified these Cush-ACAs with percentages similar to those of the general population of ACAs (8, 19). We believe that our study has two practical implications. The first consequence is that future studies concerning unenhanced CT attenuation value in adrenocortical tumors will have to integrate the functional status. The second consequence is that the clinician should anticipate a high unenhanced CT attenuation value in case of a cortisol-secreting tumor. If the unenhanced attenuation value is >10 HU, the radiologist will move on to the full washout study. Indeed, the question of malignancy remains even if we know from the beginning that this cortisol-secreting mass will be removed. The underlying issue is the best surgical approach in case of suspicious malignancy.

Yamada *et al.* (18) also report an inverse linear relationship between unenhanced CT attenuation value and the number of lipid-rich clear cells in a smaller series of 15 Cush-ACAs; however, the level of cortisol hypersecretion and Weiss score were not reported. From this

previous study, it is worth noting that for a given unenhanced CT attenuation value the density of lipid-rich cells varied widely. Of six ACAs with an unenhanced CT attenuation value close to 20 HU, the percentage of lipid-rich cells was between 10 and 55%, suggesting that, although correlated, there is wide variation. In our larger series, two of the 14 ACAs with an unenhanced CT attenuation value $\geq$30 HU did not fulfil the Weiss score criteria, suggesting poor lipid content (<25% clear cells). This discrepancy could be due to either known limitations in the measurement of unenhanced CT attenuation value (20, 21, 22) or the quantification of lipid-rich clear cells. In this regard, a recent study conducted to improve the application of the Weiss system showed that the evaluation of clear cells is subject to a certain degree of uncertainty. This Weiss score item presents inter-observer heterogeneity but also intra-observer variability (7). In the series reported by Yamada *et al*. (18) the ACAs with <25% lipid-rich clear cells had a unenhanced CT attenuation value of >17.5 HU. In our cohort, the equivalent ACAs with <25% clear cells had an attenuation value >24 HU.

The poor lipid content of cortisol-secreting ACAs could be explained by constitutive activation of the cAMP pathway, mimicking the action of ACTH. After ACTH stimulation, cultured non-functioning ACA showed characteristic changes from clear-type into compact-type cells due to a decrease in intracytoplasmic lipid droplets (23). Until recently, a small proportion of cortisol-secreting ACAs was explained by mutations in genes encoding actors of the cAMP pathway (24, 25, 26). The key role of this signaling pathway has been reinforced by recent papers reporting recurrent (35–69%) activating mutations of the protein kinase A catalytic subunit in cortisol producing ACAs (27, 28, 29, 30, 31).

It is notable that in our series, the Cush-ACA group is histologically homogeneous, with only two Weiss score criteria present: <25% clear cells and nuclear grade III or VI based on Fuhrman criteria. These results are fully in line with the conclusions of a recent paper assessing the unidimensionality of the Weiss score. Clear cells <25% and high nuclear grade are the most frequently observed items for Weiss scores 1 and 2 (32). As discussed previously, low percentage of clear cells is linked to the hypersecreting state of the adenoma. The nuclear grades were defined by Fuhrman in order of increasing nuclear size, irregularity and nucleolar prominence (15). It is suggested that in ACA the highest nuclear grade is associated with higher DNA ploidy, particularly observed in aldosterone secreting and in some cortisol-secreting adenomas with stage IV nuclear grade (33).

The Non Hyper-ACA group is histologically more heterogeneous owing to the various surgical indications: radiologically indeterminate adrenal lesions, large tumors and/or mild steroid oversecretion.

In conclusion, in our series, the vast majority of cortisol-secreting ACAs had an unenhanced CT attenuation value greater than the threshold of 10 HU, which serves to distinguish lipid-rich ACAs from other adrenal tumors. However, washout analysis remains performant in identifying this subgroup of benign adrenocortical tumors. Cush-ACAs are lipid-poor tumors, which translate histologically into a significant percentage of compact cells.

Supplementary data

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Declaration of interest

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

C Chambre, E McMurray, C Baudry, P Legmann and L Groussin contributed equally to this work.

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