True epithelial-lined cysts are rare forms of adrenal cystic lesions, the pathogenesis of which is still not fully understood. In this report we present a case of an adrenal cyst diagnosed incidentally on imaging in a 31-year-old, previously healthy, obese woman. Due to non-specific hormonal disorders and enlargement of the lesion, a right-sided laparoscopic adrenalectomy was performed. The cyst was lined predominantly by ciliated cuboidal-to-columnar, Müllerian-type epithelium, and focally by flat-to-cuboidal, mesothelium-like lining. Immunohistochemistry demonstrated a strong positive reaction in the cells of both types of lining for CKAE1/AE3, CK19, CK7 and WT1, and both had a negative reaction for CK20, CD34, Melan-A, SF1, TTF1, SMA and CDX2. The cells of the ciliated cuboidal-to-columnar epithelium were strongly positive for PAX8, ER, Ep-CAM and EMA, focally positive for PR, and were negative for calretinin, whereas the cells of the flat-to-cuboidal lining were positive for calretinin and podoplanin and showed only a weak positive response in individual cells for PAX8, EMA and Ep-CAM, but were negative for ER and PR. This is the first reported case of an adrenal ciliated epithelial cyst with Müllerian differentiation (confirmed immunohistochemically) in the English literature. The differences in morphology and immunophenotype of the two types of lining (epithelial Müllerian phenotype versus mesothelial phenotype), suggest that some adrenal epithelial cysts probably form due to metaplasia of mesothelium-derived lining. A similar mechanism may also be involved in the pathogenesis of at least some of the so-called Müllerian cysts (or inclusions) in other locations.

**Key words:** Adrenal cystic lesion, Epithelial cyst, Müllerian differentiation, Mesothelial cyst, Immunohistochemistry

**Introduction**

Adrenal cysts are rare lesions with diverse etiologies. The classification of adrenal cystic lesions was first published in the 1960’s and is still widely used, it distinguishes four classes: endothelial cysts, pseudocysts, epithelial and parasitic cysts (Abeshouse et al., 1959; Foster, 1966). Epithelial cysts account for only 2-9% of adrenal cysts (Foster, 1966; Neri and Nance, 1999; Erickson et al., 2004), their classification and pathogenesis however remain ambiguous. According to the literature, epithelial cysts are divided into three categories: true glandular or retention cysts, embryonal cysts and cystic adrenal tumors such as adenoma or pheochromocytoma (Foster, 1966; Lack, 2007). However, the latter category remains controversial, and some authors argue that cystic adrenal tumors should be classified differently, for example as pseudocysts (adrenocortical neoplasms with cystic changes or degeneration) or endothelial cysts (cystic pheochromocytoma), and only cysts with a true epithelial lining (confirmed for example by cytokeratin immunoreactivity) should be considered epithelial cysts (Erickson et al., 2004; Matsukuma et al., 2013). The pathogenesis of epithelial cysts is difficult to explain because adrenal glands do not naturally contain any
Adrenal cyst with Müllerian differentiation

structures which can form a cytokeratin positive epithelial cyst. There are some hypotheses in the literature, but little evidence concerning their histogenesis. Studies using electron microscopy and immunohistochemistry showed that some epithelial cysts are of mesothelial origin (Fukushima et al., 1995; Suh et al., 2008). However other adrenal cysts with cytokeratin positive lining reported in the literature did not have mesothelial morphology or immunohistochemical markers, and their histogenesis remains unclear (Chien et al., 2008; Limaiem et al., 2012). Herein we present the clinical and pathological findings of an adrenal epithelial cyst with Müllerian differentiation confirmed by immunohistochemical studies.

Material and methods

Patient presentation

A previously healthy, 31-year old, obese (BMI= 31) woman was admitted to hospital for diagnostic workup and management of a right adrenal mass which was discovered incidentally during an abdominal ultrasound exam. An abdominal computed tomography (CT) performed prior to hospitalization showed a lesion of the right adrenal gland measuring 40x35x30 mm with well-defined borders and a density of around 15-25 HU, with no enhancement after intravenous contrast injection, which suggested an adrenal cyst. Biochemical studies revealed ACTH and cortisol levels within reference ranges, normal circadian rhythm of cortisol secretion and a normal response to the dexamethasone suppression test. The results of 24-hour urine test for metoxycatecholamines were within normal limits. The only abnormalities were a slight elevation of DHEA-S (389.4 µg/dl, reference range: 98.8-340) and testosterone (1.98 nmol/l; reference range: 0.1-1.42) serum levels. A second CT scan (performed while hospitalised, 11 months after the first CT) revealed minor enlargement of the lesion to 45x42x30 mm. Due to the laboratory and imaging results, enlargement of the tumor, and hormonal disturbances of which the adrenal lesion as the etiology could not be ruled out, the patient was scheduled for laparoscopic adrenalectomy. During surgery a cystic mass was identified in the right adrenal gland. The lesion was successfully removed after evacuation of its liquid content. No complications were observed and the patient was discharged from hospital in good condition on the third postoperative day. Follow-up abdominal ultrasounds performed 11 and 48 months after the surgery showed no evidence of tumor recurrence. Postoperative DHEA-S, ACTH and cortisol levels 11 months after the surgery were within normal limits. The patient was alive without clinical recurrence 4 years after the surgery.

Histological process and immunohistochemistry

The surgical specimen was routinely fixed in 10% buffered neutral formalin and selected tissue samples were embedded in paraffin. Four micrometer-thick sections were taken for immunohistochemistry and for staining with hematoxylin and eosin (H&E). The steps of the immunostaining procedure (automated using a Dako autostainer) were as follows: the 4 µm formalin-fixed, paraffin-embedded sections were subjected to PT Link (DakoCytomation, Denmark) treatment in order to remove paraffin and perform high temperature antigen retrieval in EDTA Buffer pH 9.0. The endogenous peroxidase activity was blocked by incubation with 3% hydrogen peroxide for 30 minutes. After incubation primary antibodies were applied. The following antibodies were used in this study: from Dako (Glostrup, Denmark) ready-to-use (RTU) against: pan-cytokeratin (clone AE1/AE3), cytokeratin (CK) 5/6 (clone D5/16B4), CK7 (clone OV-TL12/30), CK19 (clone RCK108), CK20 (clone KS20.8), CK high molecular weight-CKHMW (clone 34BE12), epithelial membrane antigenEMA (clone E29), epithelial antigen Ep-CAM (clone Ber-Ep4), vimentin (clone V9), Wilm’s tumor 1- WT1 (clone 6F-H2), calretinin (clone DAK-calret1), podoplanin (clone: D2-40), estrogen receptor-α (clone ER clone ID5), progesterone receptor- PR (clone PgR636), smooth muscle actin- SMA (clone 1A4), melan-A (clone A103), thyroid transcription factor- TTF-1 (clone 8G7G3/1), CD34 (clone QBEnd10), CDX2 (clone DAK-CDX2); from Proteintech Group against paired box protein 8- PAX8 (cat.no 10336-1-AP, 1:1000); from Perseus Proteomics, Inc. (Tokyo, Japan) against steroidogenic factor 1/AD4BP- SF-1 (clone N1665, 1:100). Detection was performed with the dual anti-rabbit and anti-mouse EnVision FLEX High pH Detection System (DakoCytomation, Denmark) and the chromogen 3, 3’-diaminobenzidine (DakoCytomation, Denmark). Cell nuclei were counterstained with hematoxylin. Positive tissue controls were used for all antibodies (according to the manufacturer’s recommendations). Negative controls were performed by omitting the primary antibodies. Immunostained sections were then dehydrated and coverslipped in an Klinipath automatic mounting machine (Marlour, Poland).

Results

Pathologic findings

The surgical specimen consisted of an adrenal gland (with adherent adipose tissue) measuring 65x60x20 mm. Macroscopic examination revealed a thin-walled cystic lesion within the adrenal gland measuring 45x33x20 mm, its internal lining was smooth and red-brown in color; the cortex and medulla of the adrenal gland beyond the lesion were normal. Light microscopy showed a thin-walled cyst (Fig. 1A) lined predominantly by cuboidal to columnar and partly pseudostratified epithelium, without cellular atypia in which ciliated (of which some had abundant clear cytoplasm), secretary and intercalated (peg-like) cells were identified. The
described fallopian tube-like lining was consistent with Müllerian epithelium (Fig. 1B,C). There were other morphologically different areas of lining— one cell layer thick, and built of flat (only focally cuboidal) cells resembling mesothelial cells (Fig. 1B,D). In the majority of the lining, both the Müllerian-type as well as the mesothelial-like cells were located directly above adrenocortical cells or separated from them by a thin layer of connective tissue. Incomplete smooth muscle bundles were seen only focally beneath the lining. Cartilage and glands were not observed.

Immunohistochemistry

In both types of cyst lining: Müllerian-type and mesothelial-type, a strong and diffuse positive reaction was present for CKAE1/AE3 (Fig. 2A), CK19 (Fig. 2B), CK7 (Fig. 3E,e), WT1 (Fig. 3D,d) and both were negative for CK20 (Fig. 2C), SF-1 (Fig. 2D), Melan A (Fig. 2E), TTF-1 (Fig. 2F), SMA (Fig. 2G), CD34 (Fig. 2H) and CDX2 (Fig. 2I). The Müllerian-type epithelium cells showed diffuse positive reaction for PAX8 and ER (Fig. 3A,B), while the mesothelial-type lining was positive only in individual cells for PAX8 (Fig. 3a) and was negative for ER (Fig. 3b). The reaction for PR in the Müllerian lining was focally positive (Fig. 3C), but negative in the mesothelial-type lining (Fig. 3c). The flat lining showed a positive reaction for podoplanin and focally for CKHMW while in the Müllerian-type epithelium podoplanin and CKHMW were positive only focally and apically (Fig. 2L,M); additionally podoplanin positive cells were located focally in the basal layer of the Müllerian-type epithelium (Fig. 2O). There were distinct differences in immunoreactivity concerning the mesothelial marker calretinin and epithelial markers (Ep-CAM and EMA). The mesothelial-type lining was positive for calretinin (Fig. 2J), weakly and focally positive for EMA (Fig. 3F) and positive only in individual cells for Ep-CAM (Fig. 2K), the Müllerian-type lining however showed a strong reaction for Ep-CAM (Fig. 2K) and EMA (Fig. 3F), with a completely negative reaction for calretinin (Fig. 2J). Both types of lining were negative for vimentin and for CK5/6, we found only isolated, scattered positive cells in

![Fig. 1. Histological analysis (H&E staining). Adrenal thin-walled cyst at low magnification (A). The cyst was lined by two types of lining: ciliated cuboidal-to-columnar (Müllerian-type) epithelium (thick arrow) and flat-to-cuboidal lining resembling mesothelium (thin arrow) (B). The Müllerian-type epithelium (fallopian tube-like) in which ciliated, secretory and intercalated (peg-like) cells were identified (C). The second lining— one cell layer thick, built of flat (only focally cuboidal) cells resembling mesothelial cells (D). Scale bars: A, 1 mm; B, 200µm; C, D, 50 µm.](image-url)
Fig. 2. Immunohistochemical analysis of cyst lining- Müllerian-type (thick arrow) and mesothelial-type lining (thin arrow). Both types of cyst lining were positive for CKAE1/AE3 (A) and CK19 (B) and they were negative for CK20 (C), SF-1 (D), melan A (E), TTF1 (F), SMA (G), CD34 (H), CDX2 (I). The mesothelial-type lining was positive for calretinin (J), for podoplanin (L) and focally for CKHMW (M), but was mostly negative for Ep-CAM (K). The Müllerian epithelium was negative for calretinin (J), mostly negative for podoplanin (L) and CKHMW (M), but was strongly positive for Ep-CAM (K). Additionally, isolated, scattered positive cells for CK5/6 (N), podoplanin (O) and vimentin (P) were found focally in the basal layer of the tube-type epithelium. Scale bars: 50 µm.
both linings, although in the Müllerian-type epithelium the positive cells were located in the basal layer only (Fig. 3N-P). A summary of the immunohistochemistry results for both types of lining is presented in Table 1. The morphological picture of the cyst, along with the lining having focal features resembling fallopian tube epithelium and the epithelial immunophenotype showing strong nuclear positivity for PAX8, ER and locally PR allowed for the diagnosis of an epithelial adrenal cyst with Müllerian differentiation.

**Discussion**

The vast majority of cystic changes in the adrenal glands are pseudocysts (cystic changes without true lining) and endothelial cysts (lymphangiomatous and angiomatous type), which together account for 80-98% of adrenal cystic lesions (Abeshouse et al., 1959; Foster, 1966; Neri and Nance, 1999; Erickson et al., 2004). The remaining small group of changes are parasitic cysts and epithelial cysts. The last category is controversial in terms of its definition (categorization) and origin. The main debate relates primarily to the categorization of cystic adrenal tumors such as adenoma or pheochromocytoma, which according to some authors fall into the category of epithelial or epithelium-derived cysts (Sebastiano et al., 2013). According to other authors however, cystic adrenal tumors should be excluded from the group of epithelial lesions and classified as pseudocysts (eg. adrenocortical neoplasms with cystic changes or degeneration) or endothelial cysts (eg. cystic pheochromocytoma), and only cysts which have a true epithelial lining (confirmed for example by cytokeratin immunoreactivity) should be considered epithelial cysts (Erickson et al., 2004; Chien et al., 2008). Explanation of the presence within the adrenal glands of cystic changes with cytokeratin positive lining is difficult because adrenal glands do not contain any elements from which such structures can arise. Previously, some authors have postulated that epithelial retention cysts may occur in the adrenal cortex as a recapitulation of the normal embryonic development and they presented supposed transition from the normal adrenal cortex into acinar and then cyst formation as examples of glandular retention cysts (Ghandur-Mnaymneh et al., 1979). These observations were not supported by immunohistochemical studies, but in our opinion the morphological picture of the lining (cuboidal to low columnar) could match the type of cyst we are reporting. A negative response to steroidogenic factor-1 and melan A in the entire lining of the presented cyst rules out an adrenocortical cell origin (including the embryological phase) and eliminates retention cyst from the differential diagnosis.

Some epithelial cysts are probably lesions of mesothelial origin. The theory of the mesothelial origin of epithelial adrenal cysts was first presented 27 years ago. Medeiros et al. (1989) reported a cyst of the left adrenal gland, found incidentally during the autopsy of a 72-year old man, which measured 4 cm and was lined with a single layer of cells which expressed keratins. The cyst did not have the characteristics of glandular or retention cysts, nor was it a cystic variant of an adrenal neoplasm. In later publications the theory of the mesothelial origin of epithelial adrenal cysts was once again supported by electron microscopy. Fukushima et al. (1995) reported a case of a unilocular, 5.5 cm adrenal cyst in a 55-year old woman, lined by a single layer of cuboidal cells which were strongly positive for keratin, EMA and CA-125. Electron microscopy revealed many long complex microvilli and desmosomes in the cell lining suggesting their mesothelial origin. Suh et al. (2008) was the first to immunohistochemically confirm the mesothelial origin of a cyst by using two mesothelial markers: calretinin and WT1. However some reported true epithelial cysts (with cytokeratin positive epithelial lining) did not show a mesothelial origin as they were negative for mesothelial markers: calretinin and WT1. However some reported true epithelial cysts (with cytokeratin positive epithelial lining) did not show a mesothelial origin as they were negative for mesothelial markers: calretinin and WT1.

**Table 1. Summary of immunohistochemistry results for both types of lining (Müllerian-type and mesothelial-type).**

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Cuboidal-to-columnar ciliated (Müllerian-type) epithelium</th>
<th>Flat-to-columnar (mesothelial-type) lining</th>
</tr>
</thead>
<tbody>
<tr>
<td>Result</td>
<td>staining pattern</td>
<td>Result staining pattern</td>
</tr>
<tr>
<td>CKAE:AE₃</td>
<td>+ c</td>
<td>+ c</td>
</tr>
<tr>
<td>CK19</td>
<td>+ c</td>
<td>+ c</td>
</tr>
<tr>
<td>CK7</td>
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<td>WT1</td>
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<td>+ n</td>
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<tr>
<td>SF1</td>
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<td>-</td>
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<tr>
<td>melan A</td>
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<tr>
<td>CD34</td>
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<tr>
<td>CD20</td>
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<td>CDX2</td>
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<tr>
<td>TTF1</td>
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<tr>
<td>SMA</td>
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<tr>
<td>PAX8</td>
<td>+ n</td>
<td>-/+ (l) n</td>
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<tr>
<td>PR</td>
<td>+ (l)</td>
<td>n</td>
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<tr>
<td>ER</td>
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</tr>
<tr>
<td>EMA</td>
<td>+ mc</td>
<td>-/+ (l) m</td>
</tr>
<tr>
<td>Ep-CAM</td>
<td>+ m</td>
<td>-/+ (l) m</td>
</tr>
<tr>
<td>podoplanin</td>
<td>-/+ (l) m (a) (bc)</td>
<td>+ m</td>
</tr>
<tr>
<td>calretinin</td>
<td>-</td>
<td>-/+ (l) f</td>
</tr>
<tr>
<td>CK 5/6</td>
<td>-/+ (l) c (bc)</td>
<td>-/+ (l) f</td>
</tr>
<tr>
<td>CKHMW</td>
<td>-/+ (l) m (a)</td>
<td>+ (l) m</td>
</tr>
<tr>
<td>vimentin</td>
<td>-/+ (l) c (bc)</td>
<td>-/+ (l) m</td>
</tr>
</tbody>
</table>

WT1, Wilms tumor 1; SF1, steroidogenic factor 1; CKHMW, cytokeratin high molecular weight; EMA, epithelial membrane antigen; Ep-CAM, epithelial antigen; PAX8, paired box protein 8, D2-40, podoplanin; ER, estrogen receptor-α; PR, progesterone receptor; SMA, smooth muscle actin; TTF1, thyroid transcription factor 1; f, focal reaction; i, reaction in individual cells; c, cytoplasmic staining; m, membranous staining; n, nuclear staining; mc-membrano-cytoplasmic staining; a, reaction in apical surface of cells; bc, reaction in basal cells.
Fig. 3. Immunohistochemical analysis of cyst lining. The Müllerian-type lining was strongly positive for PAX8 (A), ER (B), WT1 (D), CK7 (E), EMA (F) and focally positive for PR (C). The mesothelial-type lining was positive for WT1 (d), CK7 (e), focally positive for EMA (f), weakly positive in individual cells for PAX8 (a), and negative for ER (b) and PR (c). Scale bars: 50 µm
Adrenal gland of a 70-year-old man who died due to lung cancer, the cyst was lined by flattened mesothelial-like or endothelial-like cells, which showed positive reactivity for CK7, CK19 and EMA, and were negative for CD34, D2-40, calretinin and vimentin. In the vicinity of the cyst the authors noted AHF-related intra-adrenal bile ductules with microcystic changes, the immunoprofile was similar to the cyst-lining cells which is why the authors suggested that some small epithelial cysts may develop from these types of changes.

The adrenal epithelial cyst we described in this paper was diagnosed incidentally in the right adrenal gland of a 31-year-old obese woman. Morphological examination of the surgical specimen revealed a unilocular thin-walled cyst (measuring 45 mm in the largest dimension) of the adrenal gland, which was lined by two types of epithelium: cuboidal to columnar, partly pseudostratified and ciliated, resembling fallopian tube epithelium (Müllerian-type lining) and one cell layer thick, composed of flat (in some places cuboidal) cells resembling endothelial or mesothelial cells (mesothelial-type lining). Immunohistochemistry showed a strong, diffuse positive reaction (in both types of lining) for pan-cytokeratin (AE1/AE3), CK19 and CK7, which confirmed its epithelial nature. Additionally it demonstrated a positive reaction for PAX8, ER, and focally for PR in the epithelium with tube-type morphology, allowing for the confirmation of Müllerian differentiation.

Ciliated cysts with Müllerian differentiation in locations other than the genitourinary organs or the pelvis have been rarely reported. The literature is dominated by information about Müllerian cysts in three sites other than genitourinary tract including the posterior mediastinum (Hattori, 2005; Thomas-de-Montpreville and Dulmet, 2007; Simmons et al., 2013). In our case, practically the entire perimeter of the epithelial lining of the cyst was almost directly in contact with adrenal tissue (subepithelial bands of connective tissue and muscle were seen only focally), which rather argues against a direct Müllerian duct origin of the presented case. Alternative hypotheses postulate the formation of Müllerian epithelium-lined cysts from specialized mesothelial cells of the urogenital ridge or in the process of metaplastic differentiation of misplaced mesothelium (Konishi et al., 2003; Hattori, 2005). The latter hypothesis appears to be particularly relevant in regards to the present case and offers an explanation for the etiology of a cyst with Müllerian differentiation in the adrenal gland. As previously mentioned some reported epithelial cysts were confirmed to have a mesothelial origin (Fukushima et al., 1995; Suh et al., 2008). Previously, Medeiros et al. noted that the morphological and immunologic findings of the adrenal cysts they reported were consistent with an inclusion of mesothelium, similar to epithelial cysts of the spleen (Burrig, 1988; Medeiros et al., 1989). Some authors postulate that mesothelial cells could be implanted in the adrenal gland during embryogenesis (Fukushima et al., 1995; Reiher et al., 1997). The adrenal cortex and medulla have different embryological origins. The adrenal cortex is derived from the coelomic mesoderm of the urogenital ridge, while the medulla is derived from the neural crest tissue (which derives from the ectoderm). At 7-8 weeks of fetal development chromaffin cells of the medulla migrate towards the adrenal cortex, and cortical cells are separated from the rest of the mesothelial tissue and gradually surround the medulla (Barwick et al., 2005). It can be assumed that
the mesothelial tissue might have been involved in the embryological formation of the adrenal glands. Then, under the influence of various factors (e.g. hormonal or microenvironmental) could lead to cyst formation and possibly metaplasia of the flat-to-cuboidal mesothelial lining to tube-type columnar epithelium.

The case we are reporting seems to support the theory of metaplastic Müllerian cyst formation. The cyst was lined by two morphologically different types of lining: in addition to the dominant fallopian tube-type lining, there were areas of flat-to-cuboidal mesothelial-like lining. The immunophenotype of both types of cells for a number of markers was similar. The cells in both types of lining showed a positive reaction for CKAE1/AE3, CK19, CK7, WT1, but negative reaction for SF1, Melan A, CD34, CDX2, SMA. However, there were fairly clear quantitative and qualitative differences in terms of the immunophenotype in both types of cells. The Müllerian-type epithelium showed a strong, diffuse nuclear reaction for PAX8 and Podoplanin. The differences in reactivity were seen particularly well in areas where one type of lining transitioned into the second type of lining. Vimentin staining was particularly interesting, as both types of lining were almost completely negative for vimentin, although positive cells were focally visible in the basal layer of the tube-type epithelium (mesothelial differentiation), while there was a total lack of reaction in the apical cells (mesothelial differentiation) (Fig 3d).

Since the case presented in this study shows that the pathogenesis of Müllerian cysts may also involve metaplasia, the suggestion of other authors (Thomas-de-Montpreville and Dulmet, 2007; Lee et al., 2014), to use the descriptive term "cyst with Müllerian differentiation", instead of "cysts of Müllerian origin" or "Müllerian cyst" seems fully justified.

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Jannasch O., Buschel P., Wodner C., Seidensticker M., Kuhn R., Lippert
Adrenal cyst with Müllerian differentiation


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