Female adnexal tumor of probable Wolffian origin: clinicopathological, immunohistochemical and cytofluorimetric analyses of a 22-year-old virgin. Case report

G.C. Balbi, L. Del Piano, D. Labriola, S. Visconti, A. Monteverde, M. Passaro, R. Monaco[1], A. Cardone, R. Rossiello[2], S. Panariello, L. Montone[2]

Department of Obstetrics and Gynecology, Second University of Naples (SUN)
[1]U.O. Anatomic Pathology, A.O.R.N. Cardarelli
[2]Service of Pathological Anatomy "L. Armanni", Second University of Naples (SUN), Naples (Italy)

Summary

The term female adnexal tumor of probable Wolffian origin "FATWO" designs this tumor wich arises by the rare persisting remnants of the mesonephric duct (Wolffian duct). About 40 cases have been reported in literature. Few cases of recurrence have been reported, FATWO usually shows no signs of hormonal activity. We report a case of the youngest patient affected by FATWO in October 2002. At laparotomy the left adnexa were deformed by a well-capsulated mass, totally removed and sent to the pathologist with a specimen of peritoneal fluid and of the omentum. The histological examination showed a prevalent tubular structure with focal retiform area, without intraluminal mucines. Immunohistochemical findings of the case reported are similar to those described by other authors, except for inhibin which has not been detected by us. The cytofluorimetry showed the low presence of aneuploid cells, with a very low prolifing component (< 1%).

Key words: Wolffian tumor; FATWO; Histological examination.

Introduction

The term female adnexal tumor of probable Wolffian origin 'FATWO' was first used by Kariminejad and Scully [1] to describe a tumor which showed a particular histological appearance not resembling müllerian structures. This tumor arises within the rete ovarii via rare persisting remnants of the mesonephric duct (Wolffian duct). So far about 40 cases have been reported in the literature. Since this neoplasm is so rare, it is quite hard to establish the recurrence rate [2, 3]. Although few cases of recurrence have been reported by different authors, FATWO usually have benign behaviour, showing no signs of hormonal activity. One case of FATWO with hormonal function was reported in 1995 by Inoue *et al.* [4], as an exceptional behavior of Wolffian tumors.

This study has two main purposes: to describe a case of the youngest patient affected by FATWO [5], and to verify the proliferation rate of this cell cluster through immunohistochemistry and cytofluorimetric analyses.

Case Report

A 22-year-old virgin was referred to the Gynecological Oncologic Department of the Second University of Naples (SUN) in October 2002 for left lower quadrant pain. Pelvic transrectal examination revealed a regular uterus and a painful mass on the left side. The patient had a clinical history of a lipoma of the left arm which had been surgically treated two years before. Ultrasound examination revealed a left adnexal hypoechoic mass measuring 46.3 x 33.7 mm. The uterus and right ovary were regular. No ascitic fluid was detected. Serum tumor markers were CA 125 = 15.66 U/ml (normal < 35 U/ml), CA 19.9 = 11.9 U/ml (normal < 37 U/ml), CA 15.3 = 15.13 U/ml (normal < 25 U/ml), AFP = 0.62 and CEA = 0.86.ng/ml.

At laparotomy the left adnexa was deformed by a well-capsulated mass which was totally removed and sent to the pathologist together with a specimen of peritoneal fluid and omentum. The postoperative course was uneventful.

Pathological features

The neoplasm, situated within the broad ligament, was well separated from the ovary and the tube. It was 50 x 30 x 30 mm in dimension, greyish-yellow with white areas. The cut surface was predominantly solid, without hemorrhage or necrosis, and with typical firmness. The left ovary was 30 x 20 x 10 mm, presented two little subcortical cysts and a corpus luteus. The left tube and omentum were normal. The peritoneal fluid was negative. Several tissue specimens of neoplasm were fixed in formalin and embedded in paraffin. Histological sections were stained by H&E and PAS.

Histological examination showed a prevalent tubular structure with a focal reticular pattern (Figure 1); among the tubules a moderate quantity of fibrous stroma could be detected. The tubules were narrowly structured, presented a branching, anastomozing pattern and were lined by a single layer of cuboid epithelium, with bland, round or oval shaped nuclei of small size. Chromatin was finely dispersed and occasional micronucleoli could be detected. Unicellular necrosis could be focally observed. The mitotic rate was low (2 x 10 HPF). The stroma presented areas of edema and scattered lymphoid aggregates. Investigation for mucine in neoplastic cells was negative. The basement membrane contained PAS-positive material.

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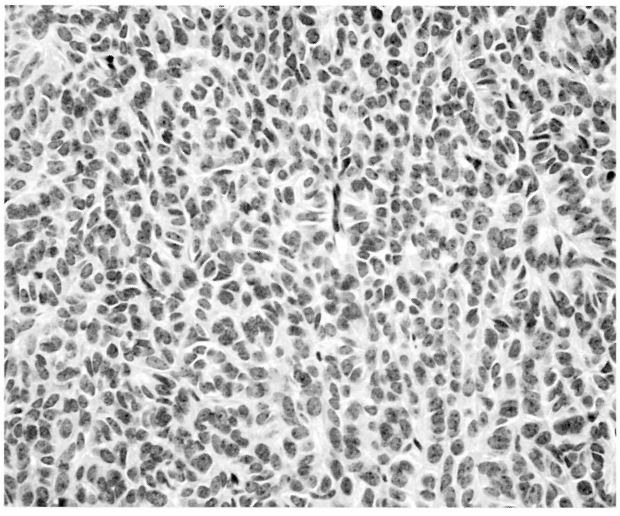

Figure 1. — The tumor shows a tubular architecture (250 x H&E).

Immunohistochemistry

Immunohistochemical methods according to Hsu *et al.* [6] were performed using the following antisera and various pretreatments of sections (Table 1). Appropriate positive tissue controls were used. Negative controls were performed by replacing a primary antibody with normal rabbit serum (1:50 DAKO).

Table 1 — *Immunohistochemical methods.*

Antibodies	Provider	Dilution
Vimentin[1]	Dako	1:50
Epithelial membrane antigen[2]	Bioptica	1:100
Pan cytokeratin (MNF 116)[1]	Bioptica	1:50
LMW cytokeratin[1]	Dako	1:50
HMW cytokeratin[1]	Dako	1:35
AE1-AE3[1]	Dako	1:50
Carcinoembryonic antigen[3]	Dako	1:50
ER[3]	Dako	1:50
PgR[3]	Dako	1:10
Alpha-inhibin[2]	Bioptica	1:50
Synaptophysin[2]	Ylem	1:20
Chromogranin[3]	Dako	1:50
Neuron-specific enolase[2]	Neomarkers	1:50
Ki-67[3]	Immunotech	1:200
p 53[3]	Dako	1.50

1) Pretreated with protease enzyme (DAKO) for 10 minutes; 2) Without pretreatment; 3) Pretreated in 0.01 M citrate buffer (pH 6) solution (DAKO) for 5 minutes in a microwave oven at 750 W.

Immunohistochemical staining was positive for vimentin, low molecular weight (LMW) cytokeratin, pan cytokeratin (MNF116) and for cytokeratin AE1/AE3. Rare positivity was detected for progesterone-receptor (PgR). Ki-67 expression was sporadic (< 5% of neoplastic cells). There was no evidence of p53 expression. Negative staining was observed for HMW cytokeratin, EMA, CEA, alpha-inhibin, synaptophysin, chromogranin, neuron-specific enolase (NSE) and ER (Figure 2).

Cytofluorimetry

According to Hedley's procedure for handling formalin fixed-paraffin embedded tissue, two samples were analyzed for DNA content by cytofluorimetry. To enrich tumor cell components, areas with prevalent non tumour tissue (muscle, connective, etc.) were partly discarded. The measurements were performed with a cytofluorimeter Coulter Epics XL, graphics and analysis by a Pentium PC provided with dedicated software (Phoenix Flow System, San Diego, CA).

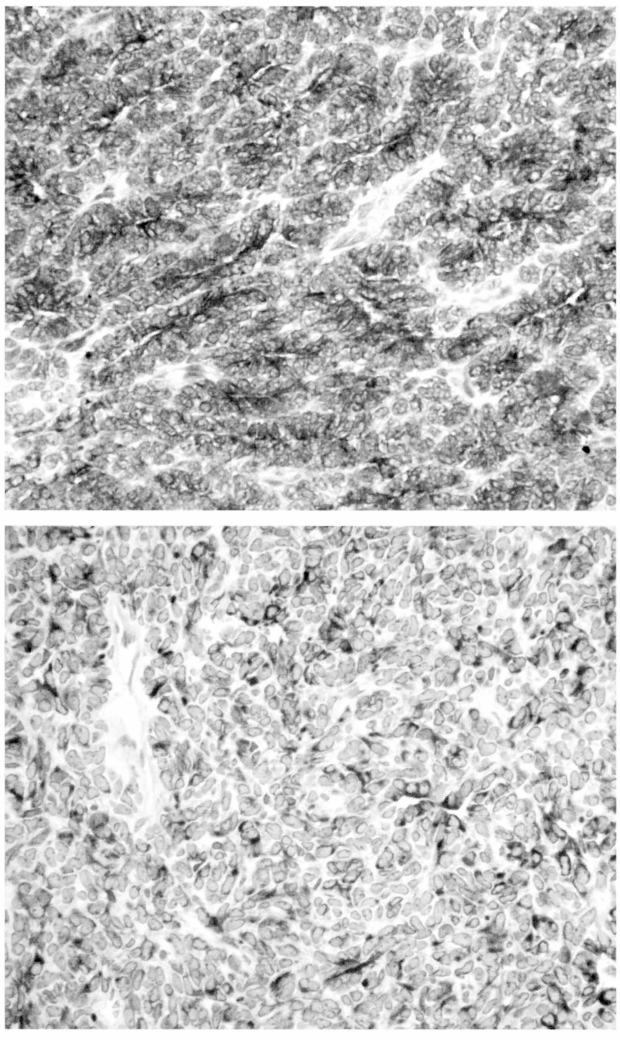

Figure 2. — Immunohistotochemical staining was positive for LMW cytokeratin (above) and vimentin (below) x 250.

According to current consensus criteria, tumors may be considered to be DNA aneuploid when two clear-cut G0-G1 peaks are visible, using normal tissue samples mixed with tumor specimens as a control of the DNA diploid peak (Bauer cytometry 1993, 14, 481).

The graphic of the analyzed cases (CV < 3) showed diploid DNA content in both tumor samples, with a very low prolifing component (< 1%), (Figure 3).

Discussion

The FATWO can originate in the typical sites of embryonic residual, such as the ovary, uterine cervix, peritoneum, broad ligament and mesosalpinx, although it is a rare tumor [8]. It is quite hard to be clinically recognized. Indeed, in the broad ligament, several tumors can be detected, such as adenomatoid and endometrioid tumors.

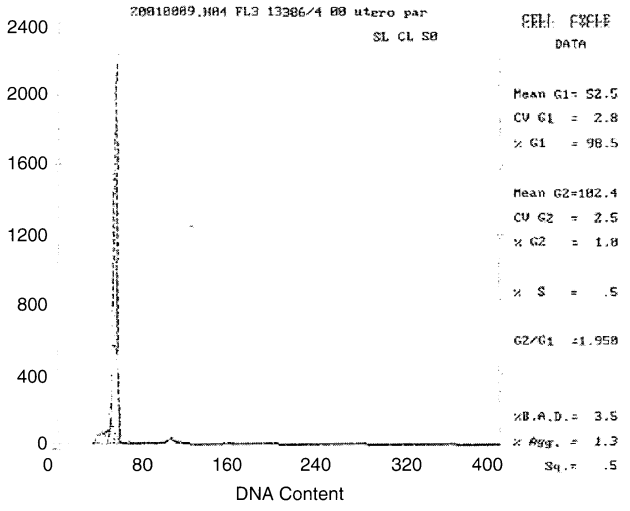

Figure 3. — Results of cytofluorimetric analysis.

Diagnostic criteria of FATWO include the anatomical site, differences from epithelial tumors of müllerian type and immunohistochemistry [7-9].

Among the several epithelial cancers, the FATWO must first be distinguished from a müllerian endometrioid tumor. Endometrioid adenocarcinoma generally presents a glandular histological growth pattern and the glands contain intraluminal mucine. In our case the tumor shows a polymorphic pattern, a diffuse tubular structure with a focal retiform area, without intraluminal mucine [10, 11].

The comparison between Wolffian tumors and granulosa cell tumors is actually easier; many cases of granulosa cell tumors reported in the literature have probably been misdiagnosed as FATWO [12]. The two tumors share the same reticular growth, but the microfollicular pattern of growth with Call-Exner bodies is typical of granulosa cell tumors. Moreover the nuclei of granulosa cell tumors are pale and regular and generally present "nuclear grooves". As reported in the literature granulosa cells tumors stain positively for vimentin, negatively for EMA and desmin and partially for cytokeratin AE1/AE3 [13].

The FATWO can also be differentiated from an adenomatoid tumor (AT) because of its morphological and immunohistochemical profile [14]. The AT consists of several gland-like structures or luminal spaces lined with flat, cuboid or low columnar cells, similar to blood vessel structures. The histogenesis of an AT is still unclear, but evidence of a mesothelial origin is on the rise. The positive expression of EMA is typical of adenomatoid tumors, while FATWOs are EMA-negative. AT stains positively for vimentin, pan cytokeratin AE 1/3 and calretinin, but negatively for Factor VIII- related antigen and S-100.

Other ovarian neoplasms were considered in the diagnostic hypothesis: Sertoli-Leydig undifferentiated cell tumors, undifferentiated carcinoma and carcinoid tumors. Sertoli cell tumors are widely associated with hormonal function, usually occur in young people, and are characterized by a papillary architecture and positive staining for alpha-inhibin. Our case did not show signs of hormonal function, had no papillary architecture, and stained negatively for inhibin. The atypical elements of undifferentiated carcinoma were absent in our case. In addition undifferentiated carcinoma co-expresses EMA and cytokeratin, while it stains negative for vimentin and alpha-inhibin. Carcinoid tumors differ from FATWOs for the morphological and immunohistochemical picture, since they generally display neuroendocrine markers, such as neuron specific enolase (NSE), synaptophysin and chromogranin.

Our findings provide immunohistochemical support for the current data in the literature: FATWOs usually express intensive staining for pan-cytokeratin (AE1/3, Ck1), CAM 5.2, vimentin, and calretinin; moderate and focal staining for cytokeratin 7 (CK7), keratin 903, epithelial membrane antigen (EMA), estrogen receptor (ER), progesterone receptor (PgR), androgen receptor, and inhibin. They do not stain for carcinoembryonic antigen (CEA) or cytokeratin 20 [7].

Immunohistochemical findings of the case reported (Table 2) are similar to those described by other authors, except for inhibin which was not detected.

As reported in the literature, CEA is always negative in cells of mesonephric origin. This staining, together with vimentin, is useful in differentiating endocervical adenocarcinoma of mesonephric origin from other endocervical adenocarcinomas which stain for CEA [8].

According to the current literature, FATWOs are generally considered as low malignant potential tumors [15] and just a few case recurrences after surgery have been reported. The low mitotic count in routinely processed sections (2 x 10 HPF) and the sporadic immunohistochemical expression of Ki-67 in our case (< 5%) confirm a very low proliferative fraction in tumor cell populations. There was no evidence in our case of p53 expression.

Cytofluorimetric analysis of this tumor, in order to determine the DNA content of the cells, was done for the first time in this study. Ideally all cells in the G1 phase take the same quantity of dye and fluorescence in a single channel. Flow cytometers are, nonetheless, not thorough and there are fewer conformational variations in DNA leading to slightly different amounts of dye being taken up. The coefficient variation (CV), allows this value to be quantified. The lower the CV, the better the DNA changes can be assessed, especially aneuploidy. In this case of a FATWO the CV was less than 3, pointing out the low presence of aneuploid cells and thus the low grade of malignancy of the neoplasm. Moreover the proliferating component was less than 1%. Although the follow-up has just been 12 months, our data allow to us to make a good prognosis for our patient.

Table 2 — *Main immunohistological markers useful in the differential diagnosis of FATWOs.*

	Female adnexal tumor of probable Wolffian origin	Adenomatoid tumor	Endometrial tumor	Undifferentiated tumor
CK AE1/3	+	+	+	+
EMA	–	+	+	+
Vimentin	+	+		–
Alfa-inhibin	±			–

+ = always positive; ± = focally positive; – = negative.

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Address reprint requests to:
G.C. BALBI, Prof.
Via Cimarosa, 84
80127 Napoli (Italy)