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Divisione Italiana della International Academy of Pathology

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ORIGINAL ARTICLE

Background. We evaluated the diagnostic accuracy of thyroid FNAC, integrated with V600E - BRAF mutational study. Herein, we report our experience using the SIAPEC cytological morphological criteria.

Methods. From September 2009 to December 2010, we performed ultrasound-guided fine needle aspiration cytology (FNAC) on 124 patients with clinical evidence of a thyroid nodule, classifying the results in five cytological categories, according to Italian Society of Pathology and Cytology (SIAPEC) consensus conference morphological criteria. In patients with indeterminate (Tir3), suggestive of malignancy (Tir4) or positive for malignancy specimens (Tir5), we obtained a new biopsy in order to study V600E BRAF status.

Patients with a diagnosis of Tir2 were assessed every six months with follow-up in the subsequent years. Patients with cytological diagnosis of Tir3, Tir4 and Tir5 underwent thyroid surgical resection with histological assessment of the lesion. Cyto-histological correlation was evaluated.

Results. We obtained the following results: Tir2 = 103 (83.1%), Tir3 = 14 (11.3%), Tir4 = 2 (1.6%); Tir5 = 5 (4%). B-RAF mutation was found on 1 Tir3, 1 Tir4 and 2 Tir5. Thyroidectomy was performed on 17 patients classified as Tir3, Tir4 and Tir5. The diagnostic specificity of FNB was of 94.5%, a sensitivity of 100%, a predictive value positive for neoplasia of 77.7 % and a predictive value of malignancy of 61.7%.

Conclusions. Diagnostic accuracy of cytology can be improved through the study of mutational status of braf gene. These additional evaluations are well studied, easy to perform and could enter in the current diagnostic procedures to optimize clinical management of thyroid nodular disease.

CASE REPORTS

Laryngeal chondrosarcomas are rare tumours that account for less than 1% of all sarcomas and originate principally from the crycoid cartilage. We report two cases: the former arising from thyroid cartilage in an 85-year-old male presenting with a palpable neck mass and hoarseness, dyspnoea and dysphagia; the other in a 54-year-old male with a mass growing from crycoid cartilage, who underwent biopsy followed by total laryngectomy. We discuss the peculiarity of the site of origin and the role of biopsy, the clinical presentation of the former case and the diagnostic and therapeutic procedures of the latter. Since it is a rare form of sarcoma arising in the larynx, we discuss the role of biopsy as a crucial although still controversial diagnostic tool.

Shadow cells are typical features of pilomatrixoma, although they have been described in other benign cutaneous tumours with characteristics of differentiation toward the hair matrix. The finding of extensive shadow cell differentiation in visceral carcinomas is otherwise unusual.

We report herein a case of uterine adenocarcinoma with extensive pilomatrixoma-like areas in a 74-year-old woman. The endometrial tumour showed an invasive poorly differentiated growth with squamous differentiation deeply extending into the myometrium intermixed with lobules of empty squamoid polyhedral cells with clear shadow like nuclei, focally exhibiting a 'ghost' appearance. The cervix, salpinges, ovaries and pelvic lymph nodes were free of disease and, taking all evidence into account, the tumour was diagnosed as poorly differentiated endometrial endometrioid adenocarcinoma (FIGO stage IB).

The recognition of an extensive pilomatrixoma-like component in a high- grade endometrioid adenocarcinoma may be important to avoid diagnostic misinterpretation with uterine metastases of malignant cutaneous pilomatrical tumours, such as pilomatrix carcinomas.

In some aspects, the terminology of fibro-osseous lesions of the head remain equivocal.

The WHO classification suggested to group cemento-ossifying fibroma and ossifying fibroma under the term ''ossifying fibroma". Based on the different age of onset, localization and risk of recurrence, two types have been described: "juvenile ossifying fibroma", with early age of onset, which needs to be treated with wide surgical resection due to the high risk of recurrence; and "adult ossifying fibroma", arising in adult patients, with low recurrence rate, properly treated by conservative surgery.

We describe a case of an "adult ossifying fibroma" of a 57-year-old woman with several relapses, for whom conservative therapy was inadequate.

We think that the "early" age of onset should not be included among the essential characteristics of ossifying fibroma with a high risk of recurrence.

A case is presented of a 58-year old man with a double multilocular cystic intratesticular tumour exhibiting the morphological features described by the WHO for diagnosis of a serous papillary cystadenoma of the ovary. We classified this tumour as the male analogue of a respective ovarian growth.

The presence of heterotopic pancreas is unusual with an estimated incidence of 0.2% of upper abdominal operations. Heterotopic pancreas occurs predominantly in the stomach, duodenum and proximal jejunum. Isolated pancreatic heterotopia of the ileum is very rare and is usually found in a Meckel's diverticulum. In most cases, these heterotopias are asymptomatic and are only incidentally detected upon pathological examination or autopsy. In this paper, the authors report two cases of pancreatic heterotopia involving, respectively, the duodenum and ileum that were fortuitously discovered on a surgical specimen and during laparotomy for unrelated causes.

High-risk human papillomaviruses (HPV) are largely implicated in the carcinogenesis of cervical carcinomas. Their role in lung carcinomas, however, is still unclear. We describe the case of 44-year-old female chain-smoker with previous HPV-related cervical cancer and a new distant tumour in the lung after many years. The histologic distinction between metastatic squamous cell carcinoma of the cervix and another primary squamous cell tumour of the lung can be difficult and has important clinical implications. The aim of our study was to investigate whether HPV was present in both the patient's cervical cancer and her subsequent primary lung cancer in order to appropriately plan therapy. We tested both the paraffin-embedded tissue of the cervical cancer and the lung cancer for HPV DNA using the Qiagen HPV Sign Genotyping Test, which detected HPV16-DNA in both tumours. The Qiagen HPV Sign Genotyping Test is a reliable method to detect HPV-DNA in tissue and cytological materials, thus making it possible to distinguish metastatic cervical carcinoma from a new primary tumour in different sites.

Pure uterine lipoma

H. Imenpour, F. Petrogalli, L. Anselmi

Pure uterine lipoma is a very rare benign mesenchymal neoplasm, and only a few cases have been reported in the literature. This is in contrast to leiomyoma, which is not only the most common neoplasm of the uterus but also one of the most common tumours in women, estimated to occur in 20-40% of women beyond the age of 30 years (AFIP) and more frequently affect postmenopausal women. We report the case of a 70-year-old woman who presented with pelvic pain and postmenopausal uterine bleeding. Pure uterine lipoma was diagnosed preoperatively by CT scan with and without contrast and confirmed postoperatively by pathological examination. Clinical and histological diagnosis of pure uterine lipoma with immunohistochemical findings are described,and the efficacy of CT in diagnosing this tumour is discussed.

GUIDELINES

Carcinoma of the exocrine pancreas: a histological report

C. Capella, L. Albarello, P. Capelli, F. Sessa, G. Zamboni

The Italian Group of Gastrointestinal Pathologists has appointed a committee to develop recommendations concerning the surgical pathology report for pancreatic cancer. The committee, composed of individuals with special expertise, wrote the recommendations, which were reviewed and approved by the Group leaders. The recommendations are divided into several areas including informative gross description, gross specimen handling, histopathologic diagnosis, immunohistochemistry, molecular findings and a checklist. The purpose of these recommendations is to provide a fully informative report for the clinician.

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ORIGINAL ARTICLE

Clinical management of thyroid nodules with indeterminate cytology: our institutional experience using SIAPEC cytological criteria and V600-BRAF test

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Key words

V600-Braf • Thyroid FNAB • Siapec Consensus Conference Molecular Biology

Summary

Background. We evaluated the diagnostic accuracy of thyroid FNAC, integrated with V600E - BRAF mutational study. Herein, we report our experience using the SIAPEC cytological morphological criteria.
Methods. From September 2009 to December 2010, we performed ultrasound-guided fine needle aspiration cytology (FNAC) on 124 patients with clinical evidence of a thyroid nodule, classifying the results in five cytological categories, according to Italian Society of Pathology and Cytology (SIAPEC) consensus conference morphological criteria. In patients with indeterminate (Tir3), suggestive of malignancy (Tir4) or positive for malignancy specimens (Tir5), we obtained a new biopsy in order to study V600E BRAF status.
Patients with a diagnosis of Tir2 were assessed every six months with follow-up in the subsequent years. Patients with cytological diagnosis of Tir3, Tir4 and Tir5 underwent thyroid surgical resection with histological assessment of the lesion. Cyto-histological correlation was evaluated.
Results. We obtained the following results: Tir2 = 103 (83.1%), Tir3 = 14 (11.3%), Tir4 = 2 (1.6%); Tir5 = 5 (4%). B-RAF mutation was found on 1 Tir3, 1 Tir4 and 2 Tir5. Thyroidectomy was performed on 17 patients classified as Tir3, Tir4 and Tir5. The diagnostic specificity of FNB was of 94.5%, a sensitivity of 100%, a predictive value positive for neoplasia of 77.7 % and a predictive value of malignancy of 61.7%.
Conclusions. Diagnostic accuracy of cytology can be improved through the study of mutational status of BRAF gene. These additional evaluations are well studied, easy to perform and could enter in the current diagnostic procedures to optimize clinical management of thyroid nodular disease.

Introduction

Thyroid nodules are very common anatomo-clinical conditions with a worldwide reported prevalence estimated to be from 15-30% of the adult population [1].
Fine needle aspiration cytology (FNAC) is considered the gold standard diagnostic test in the evaluation of a thyroid nodule. It is simple, cost-effective, readily repeatable and quick to perform [2]. Notwithstanding these considerations, the management of patients with indeterminate or suspicious FNAC specimens still remains problematic and the main topic is distinguishing nodules requiring surgical treatment from the benign ones that can be clinically observed [3-8].
Molecular biology has made significant contributions in attempting to address this issue [9 10]. Molecular testing of thyroid nodules for a panel of mutations refines the cytological diagnosis of a thyroid cell malignancy. In particular, the V600E BRAF activating point mutation is highly specific for papillary carcinoma [11 12]. The BRAF gene encodes a serine/threonine specific protein kinase, an enzyme that plays a key-role in regulating the MAP-kinase signalling pathway, which affects cell division, differentiation and secretion. The most common BRAF mutation is V600E, the replacement of a thymine with an adenine on the 1796 nucleotide, thereby obtaining a substitution Glu for Val at position 600. The RNA transcribed behaves like an oncogene. BRAF V600E is the most common genetic aberration in adult papillary thyroid cancer, found in 29-69% of cases, and is often associated with more aggressive behaviour and less differentiated tumours [13]; for all these reasons, this muta-

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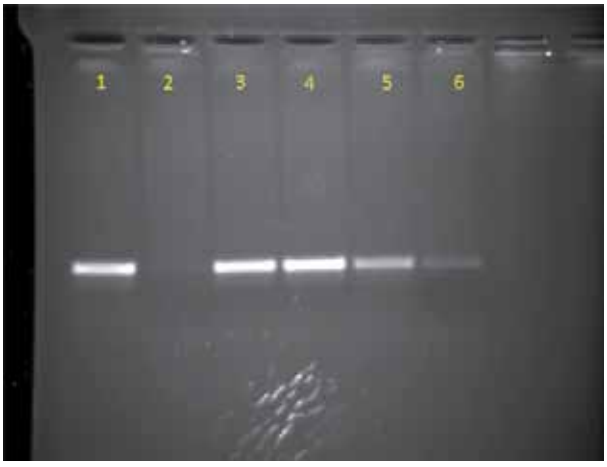
tion is considered a potential molecular biomarker for papillary thyroid cancer [14].
During our experience, we have tried to evaluate and improve the diagnostic accuracy of thyroid FNAC, using Italian morphological criteria from the Society of Pathology and Cytology (SIAPEC) [15], and integrating the V600E - BRAF mutation, in order to optimize clinical management of thyroid nodular disease.

Materials and methods

From September 2009 to December 2010, at the University Hospital of Marcianise (CE), we performed a neck ultrasonographic examination in 1296 patients with clinical evidence of a thyroid nodule. 124 patients subsequently underwent ultrasound-guided fine needle aspiration cytology (FNAC).
Ultrasound criteria. The following ultrasonographical features were considered to identify a suspicious nodule: hypoechoic appearance, irregular nodular margins, vascular pattern of the nodule from a Doppler ultrasound and the presence of intranodular microcalcifications.
Biopsy procedure. Upon assessment of patients, we acquired one aspirate for each nodule with a 23 gauge needle on a 20 ml disposable syringe mounted on a syringe holder. Cell adequacy was evaluated for each patient. The preparations were smeared onto glass slides, air dried and stained with Diff-Quick.
Cytological classification. The assessment of cytological specimens was performed according to the Italian Society of Pathology and Cytology (SIAPEC) consensus conference morphological criteria: Tir 1 - non-diagnostic specimen; Tir2 - negative for malignant cells: including colloid cystic nodule, autoimmune Hashimoto thyroiditis and granulomatous De Quervain thyroiditis; Tir3 - follicular neoplasia/atypia of indeterminate significance, considering adenomatoid hyperplasia, adenoma, follicular microinvasive carcinoma, oxyphil cell lesions. Furthermore, the pattern of a follicular or Hurthle cell neoplasm with or without atypia are included; Tir4 - suggestive for malignant neoplasm: this is a heterogeneous group of lesions characterized by few neoplastic malignant cells, numerically insufficient to make diagnosis or presenting cytological atypia insufficient to make a diagnosis; Tir5 - positive for malignancy. This group includes all cases with positive cytology (papillary, medullary, anaplastic carcinoma, lymphoma and metastatic neoplasia).
A new FNAC was obtained for nodules with non-diagnostic cytology (Tir1).
Molecular biology. In patients with a diagnosis of Tir3, Tir4 and Tir5, we performed a molecular biology test for the V600E - BRAF gene mutation (Fig. 1). DNA was extracted from cells obtained by FNAB and resuspended in 0.9% NaCl. DNA was precipitated with a salting-out method modified in our laboratory. Purity and evaluation was assessed by spectrophotometry (Biophotomaker Eppendorf), while the degree of integrity was determined with electrophoresis. Mutation study was performed by PCR – ARMS, and gene sequencing (Biosystem kit) and scanning on automatic analyzer ABI PRISM 310 (Genetic Analyzer Applied Biosystem) was employed to confirm the presence of the mutation (Fig. 1).

Fig. 1. Primers used by PCR-ARMS.

REV-COMMON 5′- ggC CAA AAT TTA ATC AgT ggA – 3′
FW-NORMALE 5′- gTg ATT TTg gTg TAg CTA CAg T- 3′
FW-MUTATO 5′ -gTg ATT TTg gTg TAg CTA CAg A- 3



ETF 2% agarose gel + ETB
Lines 1-2: B-RAF V600E mutation negative sample
Lines 3-4: B-RAF V600E –positive thyroid papillary carcinoma
Lines 5-6: papillary carcinoma variant follicular mixed

Follow-up

Patients with a diagnosis of Tir2 were followed up every six months in subsequent years. Patients with cytological diagnosis of Tir3, Tir4 and Tir5, underwent thyroid surgical resection with histological assessment of the pathology.
Cyto-histological correlation. Thyroid nodules were histologically classified and compared with cytology. Cytological diagnoses of Tir3, Tir4, Tir5 were considered correlated with surgery (true positive) when the histological diagnosis was follicular adenoma, follicular carcinoma, papillary carcinoma and other malignancies. The cytological diagnoses of Tir2, (true negative) were considered correlated when the follow-up showed that the thyroid nodule remained unchanged in size.
Statistical analysis. Sensitivity, specificity and positive predictive value of a positive cytological examination were calculated. For the calculation of diagnostic accuracy, we considered as true positive (Tp) with histological diagnosis of malignant neoplasm or follicular adenoma; we considered as false positive (Fp) with histological diagnosis of nodular goiter. Furthermore, patients with cytological diagnosis of benignant lesions were considered as true negative (Tn) only if the thyroid nodule remained unchanged over a period of several years.

Diagnostic accuracy. We estimated the diagnostic accuracy by calculating the sensitivity (Tp/(Tp+Fn); specificity (Tn/(Tn+Fp), the predictive value of neoplasia and predictive value of malignancy (Tp/Tp+Fp).

Results

Cytology and DNA extraction

Following ultrasound guided FNAC, 124 patients were classified into five diagnostic categories (Tab. I); Tir1 = 18 (14.5%); Tir2 = 86 (69.4%); Tir3 = 14 (11.3%); Tir4 = 2 (1.6 %); Tir = 4 (3.2%).
After repetition of Tir 1, 124 patients were reclassified into four categories (Tab. II); Tir 2 = 103 (83.1%), Tir 3 = 14 (11.3%), Tir 4 = 2 (1.6%), Tir 5 = 5 (4%).
All molecular tests showed positivity for thyroglobulin, and the amount of DNA extracted was greater than 80 ng.

Tab. I. Cytological diagnosis.

CYTOLOGY	SIAPEC classification	Results
Inadequate	Tir1	18 (14.5%)
Not neoplastic	Tir2	86 (69.4%)
Follicular neoplasia/atypia of indeterminate significance	Tir3	14 (11.3%)
Suggestive of malignancy	Tir4	2 (1.6%)
Positive for malignancy	Tir5	4 (3.2%)
Total		124

Tab. II. Cytology and V600 BRAF Mutation

CYTOLOGY	SIAPEC classification	V600-BRAF mutation
Not neoplastic	Tir2 = 103 (83.1%)	
Follicular suspicious	Tir3 = 14 (11.3 %)	1
Suggestive for malignancy	Tir4 = 2 (1.6%)	1
Positive for malignancy	Tir5 = 5 (4 %)	2
Total	124	4

V600 BRAF Mutation

The V600-BRAF mutation was found (Tab. III) in 1/14 of Tir3 cases (Fig. 2), 1/2 of Tir4 and in 2/5 of Tir5. The sum of Tir3, Tir4, Tir5 was 4/21 cases.

Cyto-histological correlation

17 patients with cytological diagnosis of Tir3 (10/14) and Tir-4-5 (7/7) underwent thyroidectomy and subsequent histological examination which revealed 6 papillary carcinomas,1 follicular carcinoma, 1 metastatic adenocarcinoma, 7 follicular adenomas and 2 nodular goiters (Tab. III).
Cyto-hystological correlation including adenomas, showed a correspondence for 15 patients (Tp). In two cases (Fp), we did not find a correlation between cytological diagnosis of Tir3 and Tir4, and histological diagnosis was nodular goiter.
The follow-up ultrasound, performed on patients classified TiR2 or TiR3 (5/13) did not show an increase (Fp).

Diagnostic accuracy

The diagnostic specificity of FNB was of 94.5%, sensitivity was 100%, the predictive value positive for neoplasia was 77.7 % and the predictive value of malignancy was 61.7% (Tab. III).

Discussion

Thyroid FNAC is considered a reliable pre-operative test that once seemed to be highly sensitive and specific in surgical and clinical management of thyroid nodular disease. However, it is often responsible for over- or under-diagno-

Tab. III. Cyto-histologic correlation.

Cytology	No. of patients	Not neoplastic	FA	FC	PC	Other
Not neoplastic (Tir2)	103	103 Tn				
Follicular neoplasia/atypia of indeterminate significance (Tir3)	14	2 + 4 Follow-up Fp	6 Tp	1 Tp	1 Tp	
Suggestive for malignancy (Tir4)	2		1 Tp		1 Tp	
Positive for malignancy (Tir5)	5				4 Tp	1* Tp
Totale	124	109	7	1	6	1

FA: Follicular Adenoma; FC: Follicular Carcinoma; PC: Papillary Carcinoma.

* Thyroid metastasis from gastric adenocarcinoma.

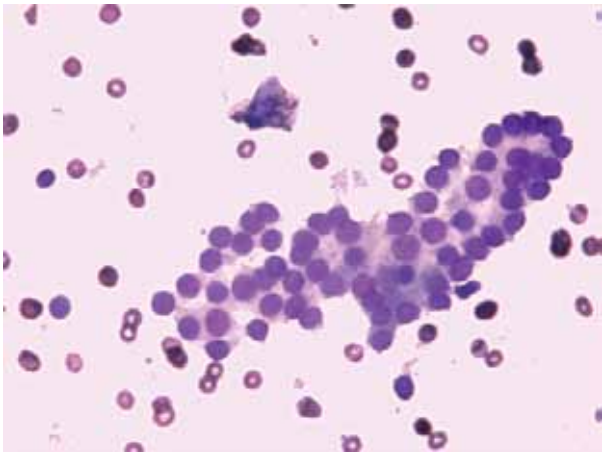
Diagnostic accuracy: True Negative = 103; True Positive:15; False Positive = 6.

Specificity = Tn/(Tn+Fp) = 94.5 %; Sensitivity = Tp/ (Tp+Fn) = 100%.

The predictive value positive for neoplasia (21/27) = 77.7%.

The predictive value positive for cancer (21/ 34) = 61.7%.

Fig. 2. Follicular variant papillary carcinoma: Diff Quick Stain.



sis because of the possible overlapping of morphological features between benignant lesions and malignancies (as for the distinction between follicular adenoma and carcinoma). In Italy, a classification proposed by Italian Society of Pathology and Cytology (SIAPEC) is currently in use that brings together follicular lesions and non-specific aspects of follicular cell atypia in a single category (TIR3 or indeterminate lesions). In recent years, bio-molecular research on malignant thyroid neoplasm helped us to refine classification of these neoplasms. Confirmation of the close relationship between the presence of the V600E BRAF mutation and papillary thyroid carcinoma potentially restricted surgical indications, thus providing an attractive bio-molecular marker in the diagnostic course of this tumour.
Based on these considerations, we decided to investigate the presence of the BRAF mutation in all specimens classified as TIR3, TIR4 and TIR5 in order to optimize clinical management within the TIR3 category.

In particular, we evaluated the diagnostic accuracy of thyroid FNAC according to SIAPEC consensus morphological criteria, validating the role of V600E-BRAF mutation on indeterminate specimens suspected or positive for papillary carcinoma.
From the analysis of 124 thyroid FNAC we obtained the following results: 83.1% of patients with non-neoplastic specimens; 11.3% of patients with indeterminate specimens (TIR3), 1.6% of patients with patients with suggestive for malignancy specimens (TIR4) and 4% positive for malignancy (TIR5).
The group of TIR2 underwent clinical and instrumental periodic follow-up, while TIR3, TIR4 and TIR5 were treated by surgery. Cyto-histological correlation showed sensitivity and specificity values of 100% and 94.4%, respectively, with a malignancy predictive value of 61%. This low value was affected by diagnoses of TIR3.
Our experience, even if performed on a small number of patients, lays the foundations for future considerations:

A) This study demonstrates that by applying the diagnostic criteria of the SIAPEC, the diagnostic sensitivity and specificity of FNAB is high;in contrast, the predictive power of a malignant disease is low, even using the BRAF mutation test.
B) The diagnostic accuracy of cytology can be improved through the study of mutational status of the BRAF gene. These additional evaluations are well characterized, easy to perform and could enter in the current diagnostic procedure.
C) Diagnosis of indeterminate specimens (TIR3) needs further investigation in order to optimize surgical management. In this regard, in future studies, it would be interesting to subdivide this category in two further subclasses using more restrictive clinical and cytological criteria to identify patients who need surgery from those that can be clinically observed.

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Case report

Role of biopsy in low-grade laryngeal chondrosarcoma: report of two cases

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Key words

Low-grade chondrosarcoma • Larynx • Biopsy • Mesenchymal tumours

Summary

Laryngeal chondrosarcomas are rare tumours that account for less than 1% of all sarcomas and originate principally from the crycoid cartilage. We report two cases: the former arising from thyroid cartilage in an 85-year-old male presenting with a palpable neck mass and hoarseness, dyspnoea and dysphagia; the other in a 54-year-old male with a mass growing from crycoid cartilage, who underwent biopsy followed by total laryngectomy. We discuss the peculiarity of the site of origin and the role of biopsy, the clinical presentation of the former case and the diagnostic and therapeutic procedures of the latter. Since it is a rare form of sarcoma arising in the larynx, we discuss the role of biopsy as a crucial although still controversial diagnostic tool.

Introduction

Chondrosarcomas of the larynx are rare cartilagineous tumours that represent less than 1% of all sarcomas [1] and approximately 0.5% of all primary laryngeal tumours [2]. Almost 600 cases have been reported in the literature [3]. The crycoid cartilage is the most commonly affected site, followed by thyroid and epiglottic cartilage. The majority of chondrosarcomas occur in middle age to elderly men and are low-grade, slowly-growing neoplasms with an indolent clinical course [1]. The treatment of choice is surgery, ranging from partial to total laryngectomy. Prognosis is generally good and regional and distant metastases are infrequent [4]. We report two cases of chondrosarcomas: a rare low-grade chondrosarcoma of thyroid cartilage causing significant dyspnoea and a laterocervical mass, and one case arising from the crycoid cartilage.

Case report

Case 1. An 85-year-old male presented to our Department for hoarseness, initial dyspnoea and worsening dysphagia. A direct laryngoscopy and biopsy were performed together with temporal tracheostomy for acute dyspnoea. The histology of the biopsy specimen showed a chondroid lesion: the specimen was characterized by well differentiated chondroid tissue, with low cellularity and bland looking chondrocytes (Fig. 1A).

Nine months later the patient was re-examined for dysphagia associated with a slowly growing right cervical mass. Clinical examination of the neck showed a painless mass on the right side near the previous tracheostomy (Fig. 1B). Fibrolaryngoscopy confirmed the presence of a laryngeal mass projecting into the lumen, causing airway stenosis. No palpable adenopathy was present in the laterocervical region.

A CT scan of the neck with non-ionic contrast and spiral technique showed a mass arising from the right side of the thyroid cartilage measuring 5 cm cranio-caudally, with stippled calcifications within and extending into hypopharynx. The mass also affected the right side of the pre-laryngeal soft-tissues, close to the hyoid bone (Fig. 1C). There was no radiological evidence of lymphadenopathy. The patient underwent total laryngectomy (Fig. 1D).

The surgical specimen consisted of the larynx with a pale grey mass, 5 cm in diameter, involving the right

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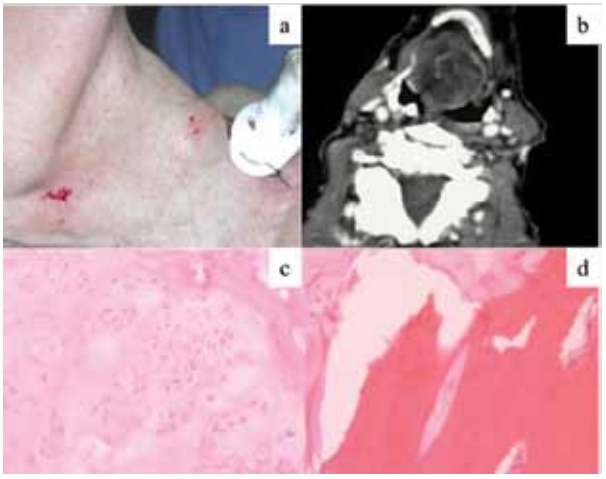
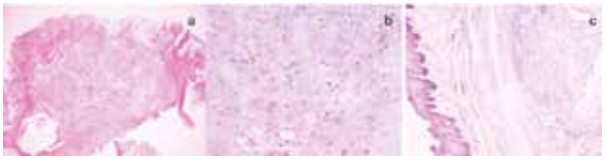
Fig. 1. (A) Cervical mass above tracheostomy. (B) CT scan showing the mass. (C) Histological aspect of low-grade chondrosarcoma showing a lobular aspect, moderate cellularity with mild nuclear atypia and hyperchromasia (10X; H/E), (D) and permeating bone marrow of the ossified thyroid cartilage (25X; H/E).



side of the thyroid cartilage, the right side of the larynx, the soft tissue upon the larynx up to the hyoid bone. The cut surface of the mass was firm and white-pale grey.
Histological examination showed a lobulated neoplasm (Fig. 1E) with borders that permeated the bone marrow of the ossified cartilage (Fig. 1F) and the pre-laryngeal soft tissue.
High power view showed moderate cellularity with small clusters or solitary chondrocytes with mild nuclear atypia and hyperchromasia. The excision margins were free of disease. The final diagnosis was low grade chondrosarcoma. The patient was free from disease three years after treatment.
Case 2. A 54-year-old patient was referred to the hospital for hoarseness. A laryngoscopy with biopsy was performed and seven biopsy samples were collected. Histology showed the chondroid nature of the lesion: the specimens were characterized by well differentiated chondroid tissue (Fig. 2A-B), with low cellularity and some binucleated, atypical chondrocytes. As the histologic report raised the suspect of malignancy, further diagnostic procedures were requested. Moreover, histopathological consultation from a specialized oncologic centre confirmed the diagnosis of low-grade chondrosarcoma.

Fig. 2. A. Biopsy specimen showing a chondroid lesion with bland nuclear atypia and some binucleated cells (B); the lesion grew beyond the laryngeal mucosa, which is free of disease. (C).



The patient underwent total laryngectomy, and the surgical specimen showed a nodular growth of the posterior wall of crycoid cartilage, 4.5 cm. in length, whitish, firm consistency and distant 1 cm from the inferior margin. No focal lesion of larynx mucosa was observed. One lymph node was isolated from the soft tissue surrounding the larynx.
Histological examination revealed a well and moderately differentiated lobulated neoplasm with pushing borders. Focal areas of ossified cartilage were present and the laryngeal mucosa did not show alterations (Fig. 2C). High power view showed moderate cellularity with small clusters or isolated chondrocytes with mild nuclear atypia and hyperchromasia. The excision margins were free of disease. The lymph node was negative. Thus, the diagnosis of the first biopsy was confirmed.
The patient is free of disease without subsequent treatment.

Discussion

Laryngeal chondrosarcoma, first described in 1816, is a rare tumour representing < 1% of all sarcomas [1] and < 0.2 % of all head and neck malignancies, and only up to 1% of all laryngeal malignant tumours, whereas it is the most common sarcoma of the larynx [2].
The posterior crycoid cartilage lamina is the site of predilection (70-75%), followed by thyroid cartilage, arythenoid cartilage and mixed location [2].
Herein, we report a case arising from crycoid cartilage and a very unusual case affecting thyroid cartilage. In the recent literature almost 20% (27/146) of all reported chondrosarcomas of the larynx were located in the thyroid cartilage [5], less than 10% (10/111) in the AFIP collection [2] and none in the Mayo Clinic collection [1]. Their pathogenesis is still unknown, but different hypotheses have been formulated.
An area of initial disordered cartilage ossification, probably induced by mechanical influence of the contracting muscles, may be associated with a pluripotential mesenchymal stem cell activation which gives rise to chondrosarcoma [2]. Hyaline cartilage usually ossifies in adults, and the age of presentation of chondrosarcoma matches with the presence of ossified cartilage and crycoid cartilage, which is the site of major mechanical muscle insult, and is also the most common site of chondrosarcoma. Ischaemic changes in a chondroma subject to mechanical trauma may also contribute to the development of chondrosarcoma [2]. Recent findings indicate that benign and malignant chondroid neoplasms of the larynx are closely related, either synchronously or metachronously [6]. Cases of larynx chondrosarcoma have also been described after Teflon injection, radiation therapy [7] and in association with other neoplasms (spindle cell sarcomatoid carcinoma) [2]. Precipitating factors of this tumour in the axial skeleton have been identified as multiple hereditary exostosis, Ollier's disease, Maffucci's syndrome, previous intravenous thoratrast contrast use,

Paget's disease of bone and chondromyxoid fibroma [8].
In our cases, the anamnesis did not disclose any previous radiotherapy or other laryngeal neoplasms. An initial disorder of cartilage ossification could not be excluded and the presence of a pre-existing chondroma in the biopsy samples showed well differentiated chondroid growth. Nevertheless, to our knowledge, there was no previous documentation of a pre-existing chondroma.
Chondrosarcoma of the larynx generally occurs in the age group ranging between the fifth and the seventh decade of life and affects males more often than females with a ratio of 3.6:1 [2].
Clinical presentation may be different, and symptoms are related to the tumour location in the larynx and size. Another peculiarity of the thyroid cartilage chondrosarcomas is that they have an expansive growth as a palpable neck mass [2].
In our first case, hoarseness was the presenting symptom associated with an initial dyspnoea: the endolaryngeal tumour growth was causing progressive airway obstruction and difficulty in breathing. This symptom is also present in chondrosarcoma of crycoid cartilage, but in our patient we also observed a slowly growing neck mass, characteristic of thyroid neoplasms. Other symptoms reported in the literature are dysphagia, dyspnoea and stridor as a consequence of impaired vocal fold mobility and/or recurrent nerve compression, eventually combined with a "mass-effect" due to endolaryngeal or exolaryngeal tumour growth.
Since laryngeal chondrosarcomas are usually low grade, in the histological examination of biopsy specimens it is often impossible to distinguish between well differentiated chondrosarcoma and chondroma if bone permeation is not present. In the thyroid cartilage case, the first biopsy showed a quite normal chondroid tissue without evident bone permeation, so that we could easily diagnose a chondroid neoplasm, but we were not certain about its malignant nature. Only the large size of the mass (5 cm) was suspect for a malignant lesion and the surgical specimen, showing bone permeation, revealed the malignant nature of the neoplasm. In the second case, the preoperative biopsy presented some atypical chondrocytes suggesting a malignant potential of the neoplasia and required further diagnostic tools. These two case descriptions highlight the controversial importance of biopsy that is apparent from an analysis of the literature. Biopsy may be inconclusive because areas of focal invasion can be missed, and because the firmness of the lesion sometimes makes it impossible to provide representative material to pathologists. At the same time, biopsy can help in the pre-operative management of the patient and guide surgical treatment. The representativeness of the tumour is crucial for a biopsy in differential diagnosis between benign and malignant phenotypes: an abundant cartilagineous matrix containing scattered small, round cartilage cells, which may show slight cellular pleomorphism and rare mitoses may be diagnostic findings to suspect a low grade neoplasm. The evidence of invasion at the growing edge, when demonstrable in the biopsy, is indicative for undoubted malignancy.
A wide surgical resection is the treatment of choice for all head and neck chondrosarcomas; nevertheless conservative resection, when necessary to preserve important structures, has resulted in long-term survival [1 4 7 9 10].
The 5-year survival rate is 70-80%, due to indolent course. Literature reports a slow progression and higher likelihood to recur rather than to cause multiple metastases. Prognostic factors are the radicality of the resection, the extension of the tumour and its histological grade [2 6].
The diagnostic role of biopsy in these low-grade tumours is still controversial, but at any rate, its importance is crucial since it is a fundamental diagnostic tool to assess the malignant nature of the lesion together with imaging diagnostics and clinical history. Although laryngeal mesenchymal lesions are notoriously difficult to biopsy, in the presence of small samples with minimal cytologic atypia of laryngeal cartilaginous neoplasms, cafe must be taken since it may be a low-grade chondrosarcoma. We expect that new biomarkers might hopefully be applied in the near future to distinguish these low grade sarcomas from their benign counterparts.

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Case report

Uterine endometrioid adenocarcinoma with extensive pilomatrixoma-like areas. A case report

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Key words

Shadow cells • Endometrial adenocarcinoma • Pilomatrixoma • Pilomatrix carcinoma

Summary

Shadow cells are typical features of pilomatrixoma, although they have been described in other benign cutaneous tumours with characteristics of differentiation toward the hair matrix. The finding of extensive shadow cell differentiation in visceral carcinomas is otherwise unusual.
We report herein a case of uterine adenocarcinoma with extensive pilomatrixoma-like areas in a 74-year-old woman. The endometrial tumour showed an invasive poorly differentiated growth with squamous differentiation deeply extending into the myometrium intermixed with lobules of empty squamoid polyhedral cells with clear shadow like nuclei, focally exhibiting a 'ghost' appearance. The cervix, salpinges, ovaries and pelvic lymph nodes were free of disease and, taking all evidence into account, the tumour was diagnosed as poorly differentiated endometrial endometrioid adenocarcinoma (FIGO stage IB).
The recognition of an extensive pilomatrixoma-like component in a high- grade endometrioid adenocarcinoma may be important to avoid diagnostic misinterpretation with uterine metastases of malignant cutaneous pilomatrical tumours, such as pilomatrix carcinomas.

Introduction

Adenocarcinoma can manifest various metaplastic features, such as squamoid components and sarcomatoid dedifferentiation consisting of non-cohesive spindle-shaped or polygonal cell components.
Shadow cell differentiation, which is otherwise typical of pilomatrixoma and is microscopically defined as the presence of cohesive empty looking "ghost" cells emerging from benign or malignant high-grade visceral tumours, has only been rarely reported [1-10].
We report herein a case of endometrial endometrioid adenocarcinoma with extensive pilomatrixoma-like areas.

Case report

A 74-year-old woman was admitted to the Division of Obstetrics and Gynecology at the Hospital of Vallecamonica for abdominal pain and a recent history of heavy vaginal bleeding. Hysteroscopic examination revealed an irregular and ill-defined endometrial mass in the uterine fundus. The patient underwent endometrial sampling with biopsy on three occasions. The material obtained from the first and the second endometrial biopsy was characterized by rare hyaline Malherbe-like aggregates of shadow cells intermingled with inflammatory elements as macrophages and neutrophils. The histopathological findings were judged inadequate for a diagnosis. A third curettage was advised and showed a high-grade endometrioid adenocarcinoma with squamous cell differentiation. Total hysterectomy, bilateral adnexectomy and bilateral iliac lymphadenectomy were performed, and the surgical specimen was sent for pathological examination.
Macroscopically, the uterus measured 5.5x4x2.5 cm. On sectioning, the uterine corpus was involved by a 4 cm slightly protruding white-greyish tumour with spotty yellowish cheesy areas extending from the fundus to the isthmus and deeply infiltrating the myometrium.
Microscopically, the lesion exhibited a predominantly solid infiltrative pattern of growth with cribriform neoplastic glands and extensive squamous cell differentiation sparing 2 mm of the outer myometrium. The neoplastic elements had abundant basophilic cytoplasm and

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hyperchromatic nuclei with obvious nucleoli. A brisk mitotic activity with some atypical figures was detected. There was no evidence of blood vessel invasion, and the cervix, salpinges, ovaries and 6 pelvic lymph nodes were histologically unremarkable. This combination of features was consistent with the diagnosis of a poorly differentiated endometrial endometrioid adenocarcinoma (FIGO stage IB). An additional interesting finding was detected, namely the presence, inside the neoplastic growth and in its infiltrative advancing front, of many areas showing pilomatrixoma-like features due to the presence of irregular sheets of empty squamoid polyhedral cells with clear shadow like nuclei, resulting in a 'ghost' appearance (Fig. 1A-B-D). These changes were frequently associated with calcifications of the stroma and a giant-cell foreign-body reaction (Fig. 1C).
Immunohistochemically, neoplastic cells showed diffuse positivity for AE1/AE3 cytokeratin and cytokeratin 7. Many of the nuclei of the adenocarcinoma cells were strongly immunoreactive to MIB-1 which recognizes Ki-67 antigen in the nucleus, and the Ki-67 index was 27%; p53 overexpression in the nucleus was observed only in a small subset of the adenocarcinoma cells (8%) (Fig. 2B-C). Immunohistochemical expression of β-catenin and E-cadherin was variably and differentially noted; no reactivity of these antibodies was detected in ghost or squamoid cells. Conversely, adenocarcinoma cells were strongly/diffusely immunoreactive to E-cadherin with intense membranous expression and a very sparse aberrant nuclear accumulation of β-catenin was also identified (Fig. 2 A-D).

Fig. 1. A) Haematoxylin and eosin-stained sections showing areas of adenocarcinoma, focally growing in solid clusters, intermixed with irregular islands of ghost cells; B) High-power view depicting shadow cells; C) Aggregates of shadow cells in the vicinity of adenocarcinomatous proliferation are surrounded by a foreign body granulomatous reaction; D) High power field of ghost cells located within the foci of adenocarcinoma with focal squamous differentiation.

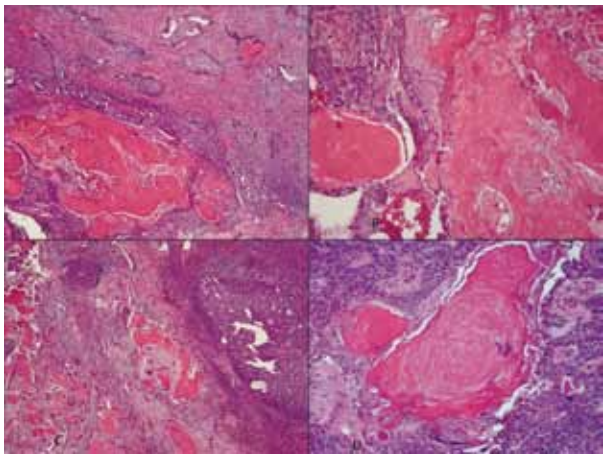
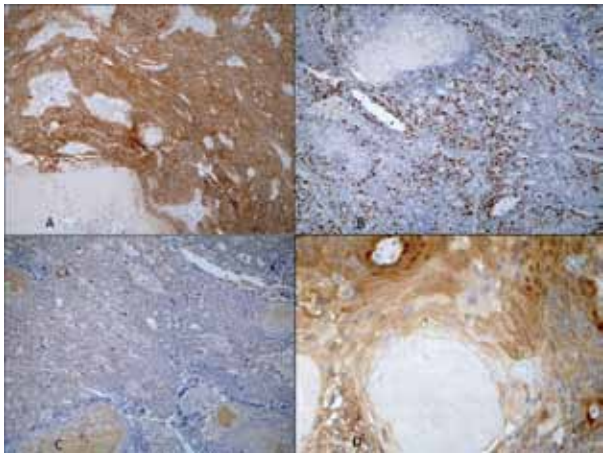


Fig. 2. A) Strong membranous E-cadherin immunoreactivity in adenocarcinoma cells sparing the pilomatrixoma-like areas; B) Many of the nuclei of tumour cells show staining for MIB-1; C) A small subset of neoplastic elements are reactive for p53; D) Sparse nuclear staining of tumour cells with antibody against β-catenin protein.



Discussion

A variable amount of shadow cells may be considered a reliable indicator of hair matrix differentiation in several types of cutaneous neoplasms and cysts, including cutaneous mixed tumours, basal cell carcinomas with matrical differentiation and proliferating trichilemmal tumours [4 11]. This matrical change can be found in the central cavity of the multiple epidermoid cysts, commonly seen in Gardner's syndrome [4 12]. The finding of shadow cells is particularly typical of pilomatrixoma which is a common skin benign neoplasm, with histological differentiation towards hair matrix (basaloid cells) and hair cortex (shadow cells). These tumours are generally located on the scalp and neck region, usually occurring in children and teenagers. Malignant forms, in adult patients and generally on the same sites, are occasionally reported as pilomatrix carcinoma, first recognized in 1980 by Lopansri and Mihm [13]. About 90 cases of this entity have been described in the literature to date [11-16]. Important criteria in establishing malignancy remain irregular and downward infiltrative growth toward the subcutaneous fat and deeper structures; marked cellular atypia with enlarged vesicular, sometimes pleomorphic nuclei, high mitotic activity with abnormal mitotic figures; foci of tumour necrosis; desmoplastic stromal reaction; vascular and perineural invasion [11]. The biological behaviour of pilomatrix carcinoma is unpredictable and in 12 published cases the tumour had aggressive behaviour and produced visceral metastases, usually to the lung and regional lymph nodes and rarely to bones, liver or brain [12 14]. The immunohistochemical reactions have shown that both benign pilomatrixomas and malignant forms diffusely expressed nuclear, cytoplasmic and membranous β-catenin [11 12 14 17]. Accumulation of nuclear β-catenin was found to be the only result of activating mutations of exon 3 of the *CINNB1* gene, which is responsible for the proteolytic degradation of β-catenin, leading to activation of the *WNT* signaling pathway. Genetic studies will be necessary to further elucidate the

specific role of activated β-catenin in the development and progression of appendageal neoplasms with matrical differentiation [12].

Shadow cells in extracutaneous anatomic sites might be better classified into specific tissue-based diagnostic categories; a small number of extracutaneous (visceral) pilomatrixomas or pilomatrixoma-like tumours have been reported probably developing from mature teratomas with ectodermal differentiation [1 4 6].

Extremely rare cases of extracutaneous (visceral) carcinomas with shadow cells have been reported including endometrial adenocarcinoma with squamous differentiation [9 10], uterine malignant mixed Müllerian tumour [7], adenosquamous carcinoma of the colon [9 10], endometrioid adenosquamous carcinoma of the ovary [3 5], transitional cell carcinoma of the bladder [10], squamous carcinoma of the lung [2], basaloid carcinoma of the anorectal region and small cell neuroendocrine carcinoma of the gallbladder [8]. Shadow cells have also been observed in atypical endometrial hyperplasia [8 18]. In 1995, Zámečnik and Michal [9] first reported shadow cell differentiation in 3 patients with endometrial adenocarcinoma, 2 patients with colonic adenocarcinoma and 1 with atypical endometrial hyperplasia. Later, these authors, in two more recent reports, stressed that the finding of squamoid shadow cells can be observed most frequently in uterine endometrioid adenocarcinoma; if systematically searched for, shadow cell differentiation is found in 6% of all endometrial carcinomas [8 10].

In the case under study, the histological resemblance to pilomatrixoma and pilomatrix carcinoma is not limited to the simple presence of shadow cells, but includes secondary changes such as calcifications and giant-cell foreign-body reaction.

The extensive presence of shadow cells in a uterine endometrioid adenocarcinoma could represent a potential diagnostic pitfall, and their detection in the context of a carcinomatous proliferation can pose problems with differential diagnostics, especially with visceral metastases from pilomatrix carcinomas of the skin or even other sites [2 5 8]. Some histologic features – irregular glandular tumour aggregations, mucin secretions – permit the exclusion of similar neoplasms, but immunostainings can be helpful in some problematic cases. In particular, adnexal skin carcinomas and their metastases usually express p63, CK5/6 or podoplanin, whereas visceral carcinomas with shadow cells normally fail to express these antigens [5]. In the present case, the dim nuclear expression of β-catenin in the uterine neoplasm supports the diagnosis of uterine adenocarcinoma, but in a recently reported work no difference of expression of β-catenin was observed in an ovarian carcinoma with shadow cells and some pilomatrixomas [5].

In summary, we present a rare case of uterine endometrioid adenocarcinoma with extensive pilomatrixoma-like areas. The diagnosis of this variant of adenocarcinoma may be challenging in a limited endometrial biopsy specimen, and the presence of lobules of Malherbe-like cells alone should prompt one to search for a more sinister component to confirm the diagnosis of this tumour [7]. Ultimately, the recognition of a shadow cell component in an otherwise typical uterine endometrioid adenocarcinoma may not be merely an academic exercise, and the pathologist should be aware that this rare type of differentiation may occur in visceral carcinomas in order to avoid diagnostic confusion with metastases of malignant cutaneous pilomatrical tumours.

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Case report

Recurrent ossifying fibroma of the maxillary sinus in an adult patient

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Key words

Ossifying fibroma • Fibro-osseous dysplasias • Odontogenic fibro-osseous lesions • Cemento-ossifying fibroma • Maxillary sinus

Summary

In some aspects, the terminology of fibro-osseous lesions of the head remain equivocal.
The WHO classification suggested to group cemento-ossifying fibroma and ossifying fibroma under the term ''ossifying fibroma". Based on the different age of onset, localization and risk of recurrence, two types have been described: "juvenile ossifying fibroma", with early age of onset, which needs to be treated with wide surgical resection due to the high risk of recurrence; and "adult ossifying fibroma", arising in adult patients, with low recurrence rate, properly treated by conservative surgery.
We describe a case of an "adult ossifying fibroma" of a 57-year-old woman with several relapses, for whom conservative therapy was inadequate.
We think that the "early" age of onset should not be included among the essential characteristics of ossifying fibroma with a high risk of recurrence.

Introduction

Fibro-osseous dysplasias of the head are rare diseases belonging to an array of tumours or tumour-like lesions of fibro-osseous tissue. Several benign fibro-osseous tissue lesions show similar histological features, but very different behaviour. In 1992, the World Health Organization (WHO) divided odontogenic fibro-osseous lesions into two groups: non-neoplastic osseous lesions, to which fibrous dysplasia belong, and osteogenic tumours, comprising cemento-ossifying fibroma (COF) and ossifying fibroma (OF) [1]. In 2005, the WHO classification [2] suggested grouping COF and OF under the term ''ossifying fibroma" (OF) due to their histological similarities, with the term COF to be abandoned. Radiologically, OF is distinguished from fibrous dysplasia for its clearly circumscribed nature, presenting as a concentrically expanding, solitary mass with bone density. Clinically, OF is usually painless in the early stages and shows unilateral swelling, often with a slow growth and erosion of the surrounding bone. Its radiologic appearance is characterized by radio-lucent lesions with or without a sclerotic border, mixed with radio-opaque areas. Two forms of ossifying fibroma have been described: an adult form (known as classic OF, cementifyng fibroma and COF) and a juvenile form (including psammomatoid juvenile OF and trabecular juvenile OF).
Juvenile OF arises in the orbit or nasal sinuses in 90% of cases [3], in contrast with classical OF which generally arises in the mandible and is usually well encapsulated, so it can be shelled out from its bed with ease. Due to its increased aggressiveness, treatment for the juvenile form consists in wide surgical resection, with 25-28% of post-operative recurrences [4-6]. Conservative resection is preferred for the adult form, for which only 5% of post-operative recurrences have been reported [7]. We describe a case of OF in an adult woman, which due to its morphological features and age of the patient could be classified as "adult ossifying fibroma"; considering its location, aggressiveness and multiple recurrences, it however shows similarities with "juvenile" or "aggressive" ossifying fibroma.

Case report

In January 2009, a 57-year-old woman with a clinical history of chronic sinusitis was evaluated for the appearance of painful and widespread left hemi-facial swelling with normal overlying skin, associated with fever, release of

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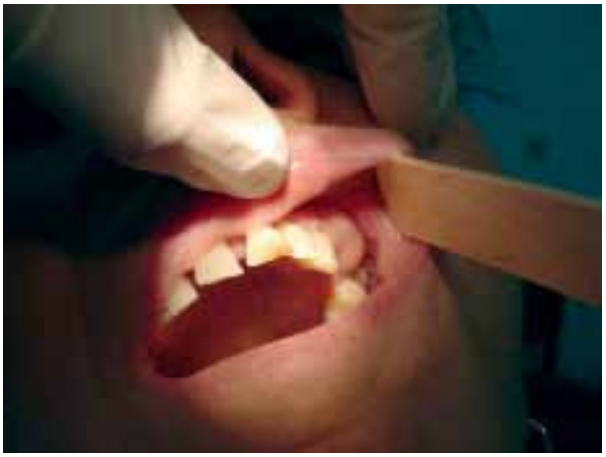
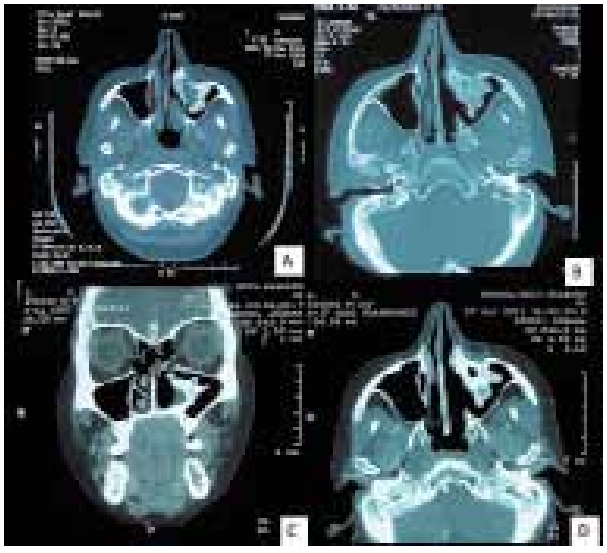
Fig. 1. Swelling of the upper left gingival fornix.



fetid purulent exudates, cacosmia and swelling of the upper left gingival fornix (Fig. 1). In 2008, the patient had undergone Caldwell-Luc surgery of the left maxillary sinus in another hospital for recurrent symptoms lasting about 4 years, with about 10 episodes of exacerbation. The histological result was not available. After surgery, she reported about a year of well-being. At admission, anterior rhinoscopy showed a lesion of bone consistency covered with normal-appearing mucosa occupying the lower portion of the left nasal cavity. Maxillo-facial computed tomography showed an uncapsulated expansive and erosive lesion occupying inferiorly the left nasal fossa and part of left maxillary sinus. It was characterized by the predominance of hyperdense areas mixed with hypodense ones, not dissociable from the front wall of the maxillary sinus, which appeared infiltrated and partially eroded with focal areas of reactive bone formation (Fig. 2a). The right maxillary sinus, the sphenoidal and frontal sinuses and the ethmoidal cells were regularly pneumatized. The patient underwent endoscopic re-excision of the lesion and the specimen was sent for the intraoperative frozen section examination. The histological specimen showed fragments of respiratory mucosa with chronic nonspecific inflammatory infiltration, including several thick wall vessels and surface fragments of bone tissue. Some consisted of lamellar bone trabeculae with an arciform or a "Y" silhouette and with osteoblastic rimming (Fig. 3 a, b, c). Others appeared as necrotic bone fragments, with strongly basophilic staining (Fig. 3d), surrounded by a fibrous stroma with loose cellularity. A diagnosis of "adult type" fibro-osseous benign lesion was made and, in keeping with the radiological demarcation from the surrounding bone and the presence of an osteoblastic rimming, a diagnosis of "ossifying fibroma" was suggested. The patient was free of symptoms for about one year, but later reported the recurrence of painful swelling in the left maxillary region, associated with intermittent fever. CT showed the recurrence of the heterogeneous density lesion, causing bone loss at the maxillary sinus floor, involving the underlying alveolar bone and extending into the sinus with a calcific

Fig. 2. CT scan. a) Lesion with predominance of hyperdense areas mixed with hypodense areas, not-dissociable from the front wall of the maxillary sinus. b) Lesion that causes bone loss at the maxillary sinus floor, involving the underlying alveolar bone and extending into the sinus with a calcific and lobed appearance. c, d) Lesion showing bone tissue density areas that were prevalent on soft tissue areas.



and lobed appearance (Fig. 2b). This new formation was removed completely with a combined endoscopic transnasal approach and through the canine fossa. Histologically, the surgical specimen was overall similar to the previous one, consisting of respiratory mucosa and fragment of fibrous tissue encompassing some bony plates with "Y" or "arciform" silhouette, basophilic concretions and fragments of necrotic bone lamellae. After about eight months of well-being, the patient returned to our observation for the recurrence of such painful swelling of the jaw and left upper gingival fornix. CT showed the reappearance of newly formed tissue with irregular margins and a maximum transverse diameter of 3.2 cm. Heterogeneous density was still evident, but the bone tissue density areas were now prevalent on the soft tissue areas (Fig. 2 c, d). The patient refused radical hemi-maxillectomy surgery and was again subjected to complete removal of the lesion using a trans-canine fossa approach with curettage of implantation sites of the lesion. The bone tissue presented again as well organized bone lamellae, with rare basophilic and necrotic areas. The stroma showed a more dense cellular proliferation of spindle-shaped cells (Fig. 4 a, b). After only three months the patient presented again a painful swelling of the region. CT confirmed the recurrence, but the patient refused further surgery and at the time of writing is affected by the disease.

Discussion

The terminology of tumour or tumour-like lesions of fibro-osseous tissue remains somewhat equivocal. In

Fig. 3. Histological features of the first recurrence. a, b, c) Fragments of respiratory mucosa with chronic nonspecific inflammatory infiltration, including several thick wall vessels and surface mature lamellar bone trabeculae with an arciform or a "Y" silhouette and with an osteoblastic rimming *(Haematoxylin-eosin staining; original magnification: 100x).* d) necrotic bone fragments, with strongly basophilic staining surrounded by a fibrous stroma, with loose cellularity. *(Haematoxylin-eosin staining; original magnification: 100x)*

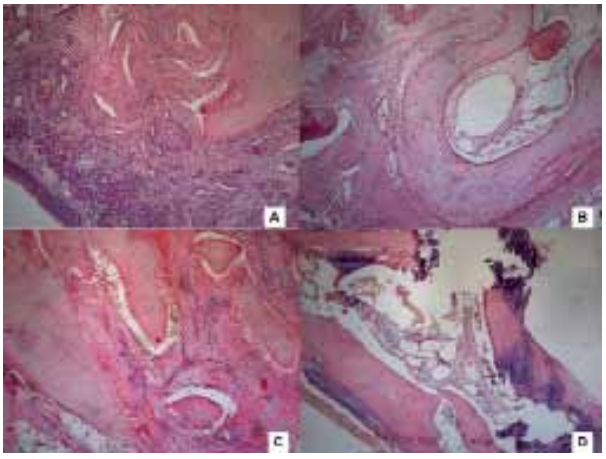
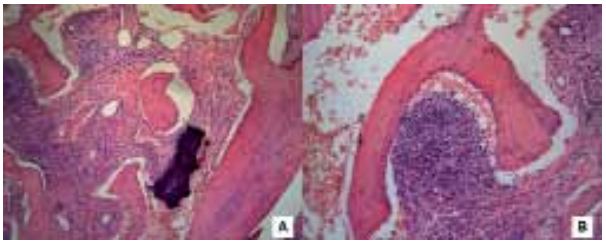


Fig. 4. Histological features of the second recurrence. a, b) Mature, well organized bone lamellae, with rare basophilic and necrotic areas and dense cellular proliferation of spindle-shaped cells in the stroma *(haematoxylin-eosin staining; original magnification: A = 100x; B = 200X)*



2005, the WHO [2] defined OF as " a well-demarcated lesion composed of fibrocellular tissue and mineralized material of varying appearances".

In this classification, the terms "cementifying" and "cemento-ossifying" fibroma, are reported as synonyms of OF, while "active" and "aggressive" ossifying fibroma are reported as synonyms of the "juvenile ossifying fibroma", the histologic variants of OF.

The previous WHO classification (1992) [1] defined "juvenile OF" as a lesion with aggressive growth in patients under the age of 15. Toro [8] summarized the essential characteristics of juvenile OF as follows: early age of onset, bone pattern, high tendency to recurrence and aggressive local behaviour. OF is believed to originate from the periodontal membrane and its presence in the paranasal sinuses could be explained by hypothesizing the origin from primitive mesenchymal cell rest [9]. The tumour grows slowly, with an aggressive character causing erosion of the surrounding bones by a compressive mechanism. In the paranasal sinuses, there is little resistance of the surrounding hard bones and the lesion continues to grow giving rise to the appearance of clinical signs [10]. The most common symptoms are sinusitis, nasal obstruction, cacosmia, facial swelling, maxillary pain, headache, fever, visual disturbance, exophthalmos and proptosis if there is orbital involvement. Radiologically, the lesion is usually composed of well circumscribed, heterogeneous tissue, characterized by hyperdense calcified matrix associated with hypodense tissue, depending on the prevalence of bone or fibrous tissue. Margins are usually well defined and can be surrounded by sclerotic tissue. The invasion of surrounding tissues is related to rapid growth creates a rarefaction of margins. The treatment is purely surgical, either through a conservative endoscopic approach, or through highly radical craniofacial resection. Sciubba and Younai [11] reported the enucleation or curettage of the lesion as the initial treatment of choice and no recurrence was found after surgical excision in this series. Chang et al. [12] supported this concept, considering "initial tissue-sparing surgery" usually adequate and curative. Noteworthy, a different treatment has been advised for the two different forms of OF, consisting in wide surgical resection for the "juvenile" form, which is more aggressive, with 25-28% of post-operative recurrences [4-6], whereas a conservative resection is preferred for the "adult" form, for which only 5% of post-operative recurrences have been reported [7]. Juvenile OF affects children under the age of 15 in 80% of cases [5] and arises in the orbit or paranasal sinuses in 90% of cases [3], in contrast with classical OF which generally arises in the mandible of adult patients. In our case, the circumscribed nature of the lesion aids in differential diagnosis with fibrous dysplasia, leading to a diagnosis of OF. Moreover, despite the adult age, the occurrence in the paranasal sinus, the lack of a capsule with destruction of surrounding bone and the numerous recurrences after conservative surgery would suggest an aggressive form with a behaviour similar to the "juvenile" form. Thus, even if some studies favour conservative surgery for lesions in adult patients [8], we think that in our case, en bloc resection or partial resection of the jaw would have been preferable, at the time of the first relapse, to avoid or minimize the chance of further recurrences.

Conclusion

In order to establish correct treatment, we believe that the criterion of "early age of onset", highlighted previously [1 8], should not be included among the essential characteristics of OF with a high risk of recurrence, needing a more aggressive surgery. In this setting, in fact, the terms "juvenile" and "adult" may be misleading for appropriate management. As they are infrequent lesions and lack clearly defined histological characteristics, close collaboration between radiologist, surgeon and pathologist is necessary.

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Case Report

Intraparenchymal serous papillary cystadenoma of the testis: A case report

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Key words

Testis • Serous papillary cystadenoma • Serous papillary cystic tumor • Testicular neoplasms • Rare tumors

Summary

A case is presented of a 58-year old man with a double multilocular cystic intratesticular tumour exhibiting the morphological features described by the WHO for diagnosis of a serous papillary cystadenoma of the ovary. We classified this tumour as the male analogue of a respective ovarian growth.

Clinical case

A 58-year-old male patient with an occasional finding of two separate intratesticular neoformations on the left testicle; ultrasonography showed two intratesticular circumscribed cystic masses, with hyperechogenic areas within and normal surrounding parenchyma: after ultrasound examination, a seminomatous nature was suspected. The patient was subjected to intervention of orchiectomy, and the surgical specimen was sent to for histological examination.

Materials and methods

The surgical specimens, fixed in 10% buffered formalin, were treated routinely with inclusion in paraffin. Serial sections were stained with haematoxylin-eosin.

Pathological findings

Macroscopic/pathological examination of the surgical specimen (consisting of testes and testicular appendages) had a total weight of 58 gm and were 7.5 x 4 x 3 cm in size. When cut, the testis (4.5 x 3 cm) showed two intraparenchymal, pericapsular, formations that were circumscribed, whitish, cystic in appearance, that were 0.8 x 0.6 cm and. 0.6 x 0.3 cm.

Microscopic examination of testicular sections showed, at the level of the tunica albuginea and in the intraparenchymal region, two separate multiloculated cysts (Fig. 1), which were adjacent but not communicating with each other, containing clear fluid. These formations had a sclero-hyaline wall (Fig. 2) containing focal calcific concretions with concentric laminations (psammoma bodies) (Fig. 3), and an epithelial lining almost entirely of ciliated columnar type (Fig. 4), mono- and pseudostratified, with only aspects of zonal pluristratification. There were endoluminal micropapillary projections originating from the cyst wall, with a stroma-vascular axis, and protruding into the cavity (Fig. 5). There was sporadic evidence of cytological atypia and no mitoses. The morphological findings suggested a diagnosis of papillary serous cystadenoma with focal cytologic atypia. The epididymis, testicular annexes and margins of resection are free.

Discussion

Testicular neoplasms resembling ovarian serous tumours are rare [7 12], and tumours reminiscent of Müllerian epithelial tumors of the ovary have been reported outside of the ovary including in the pancreas and paratesticular region [3 5]. The histogenesis of ovarian-type epithelial tumours of the testis and paratestis remains speculative with favoured theories including Müllerian metaplasia

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Fig. 1. Multiloculated cyst in the intraparenchimal region of the testis.

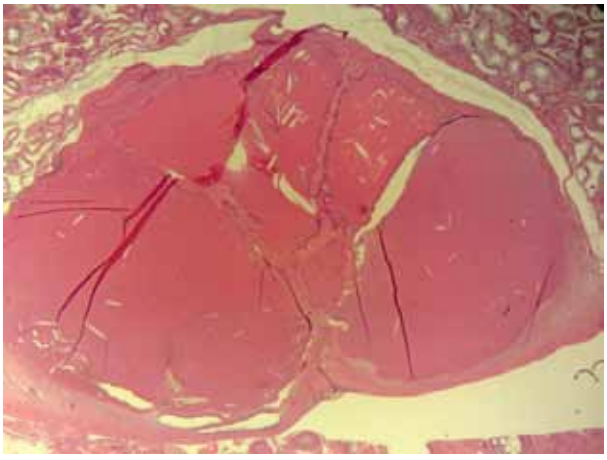


Fig. 2. Cyst: sclero-hyaline wall.

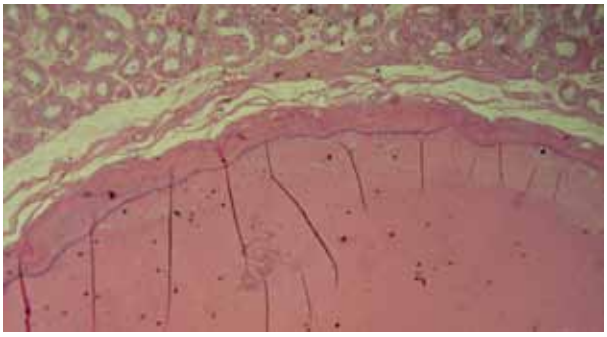


Fig. 3. The cyst wall contains focal calcific concretions.

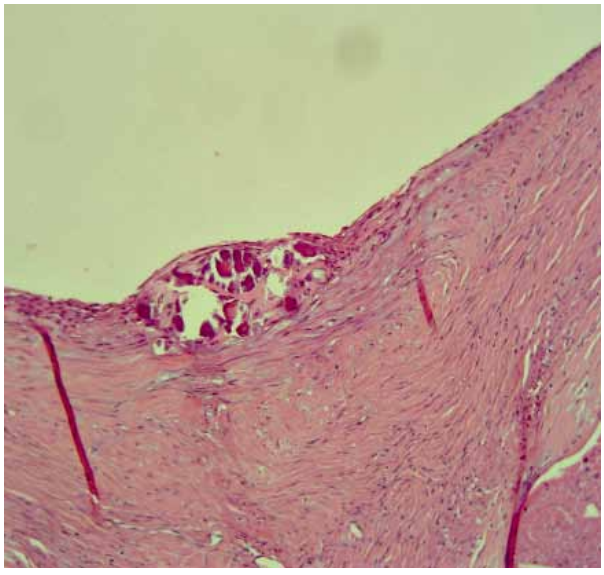


Fig. 4. A cyst lined by columnar ciliated epithelium.

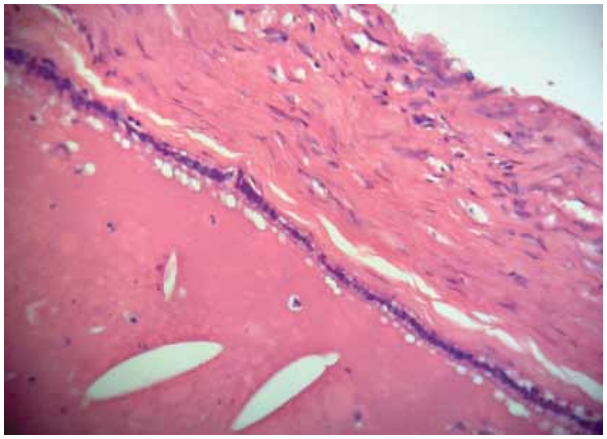
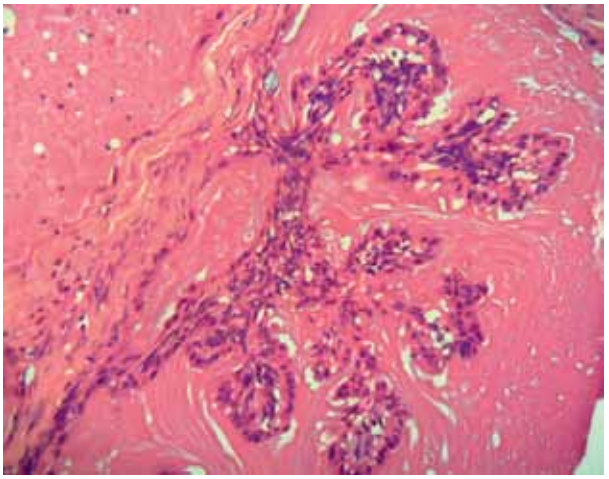


Fig. 5. Cyst: endoluminal micropapillary projections.



of the tunica vaginalis and originating from Müllerian rests in paratesticular soft tissue or the appendicular testis (based on many tumours being centered on the epididymo-testicular groove). Intratesticular tumours may develop from mesothelial inclusions or, particularly in the case of mucinous tumours, represent monodermal teratomas; in the case of the latter, the absence of associated intratubular germ cell neoplasia suggests a pathogenetic paradigm that is different from most testicular germ cell tumours, and perhaps similar to that suggested for testicular dermoid cysts.

Papillary cystadenoma of the ovarian type is an epithelial tumour that originates in the tunica vaginalis testis [4], and normally the cells are derived from metaplasia of the Müllerian mesothelium. It can be located within the testicular parenchyma due to inclusions of the mesothelium during the embryonic period. Injury, rare in this period,

could have originated from invagination of the tunica albuginea of the testis and would be similar to endosalpingiosis in the ovaries. Macroscopically, the tumour may be either singular or multiple [5 8], exophytic or papillary, cystic, solid and mostly small. It appears, upon cutting, unilocular [9] or multiloculated, with serous, mucous or haemorrhagic content. By microscopic examination, the lesion has several aspects: solid, papillary, mixed, covered with ciliated columnar epithelium type, simple or stratified mucus, or cubic type, transitional, etc. The malignant forms [10 11] are different from those from benign and borderline [2 6] for the presence of cellular atypia, necrosis and stromal invasion.

Morphologically, the most important differential diagnosis was mesothelioma arising from tunica vaginalis [1]. The latter is histologically characterized by features such as: testes of small size, low cellularity, type of surface epithelium with cubic monostratified eosinophilic cytoplasm and absence of psammoma bodies.

The treatment of choice is radical orchiectomy, and the clinical course of our patient was benign.

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Case report

Pancreatic heterotopia of the small intestine: two case reports

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Key words

Pancreatic heterotopia • Small intestine • Ileum • Duodenum

Summary

The presence of heterotopic pancreas is unusual with an estimated incidence of 0.2% of upper abdominal operations. Heterotopic pancreas occurs predominantly in the stomach, duodenum and proximal jejunum. Isolated pancreatic heterotopia of the ileum is very rare and is usually found in a Meckel's diverticulum. In most cases, these heterotopias are asymptomatic and are only incidentally detected upon pathological examination or autopsy. In this paper, the authors report two cases of pancreatic heterotopia involving, respectively, the duodenum and ileum that were fortuitously discovered on a surgical specimen and during laparotomy for unrelated causes.

Introduction

Heterotopic pancreas (HP) is defined as pancreatic tissue that lacks an anatomic or vascular communication with the normal body of the pancreas [1-3]. Although HP can occur throughout the entire gastrointestinal tract, it is most commonly found in the stomach (25%-38%), duodenum (17%-36%) and jejunum (15%-21%) [4]. Rare cases have described HP in the ileum, oesophagus, biliary tract, gallbladder, spleen and mesentery. In most cases, HP is asymptomatic and is only incidentally detected upon pathological examination or autopsy. In this paper, the authors report two cases of HP involving, respectively, the duodenum and ileum that were fortuitously discovered on a surgical specimen and during laparotomy for unrelated causes.

Clinical history

Case 1

A 52-year-old previously healthy male patient presented with a history of abdominal pain, and significant weight loss over the past two months. On examination, the patient's vital signs were stable and showed signs of jaundice. Abdominal examination revealed epigastric tenderness. Laboratory studies showed elevated bilirubin, alkaline phosphatase levels and moderately elevated serum carbohydrate antigen 19-9 (Ca 19-9). Abdominal ultrasonography revealed a hypoechoic lesion of the head of the pancreas. Abdominal computed tomography (CT) scan demonstrated a heterogeneous, hypodense mass of the head of the pancreas measuring 3 cm in diameter (Fig. 1). The patient underwent pancreaticoduodenectomy. Macroscopically, the pancreatic tumour measured 2.7 × 2.2 cm and invaded the duodenal wall (Fig. 2a). In addition, there was a nodular lesion involving the serosa of the duodenum measuring 1 cm in diameter (Fig. 2b). On cut sections, this nodule was yellow in colour with a lobulated appearance reminiscent of pancreatic tissue (Fig. 3). Histological examination showed that the pancreatic tumour corresponded to a well-differentiated ductal adenocarcinoma. The second lesion involving the duodenal serosa displayed pancreatic lobules with acini and ducts, but there was no evidence of islets of Langerhans. The final pathological diagnosis was HP of the duodenum type II Heinrich. Unfortunately, the patient died two weeks postoperatively.

Case 2

A 33-year-old woman with a medical history significant for autoimmune hepatitis was admitted for liver trans-

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Fig. 1. Case 1: Abdominal computed tomography (CT) scan demonstrating a heterogeneous, hypodense mass of the head of the pancreas.

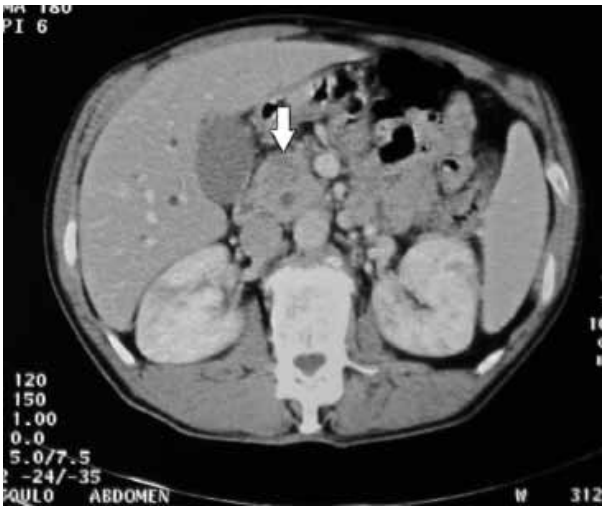
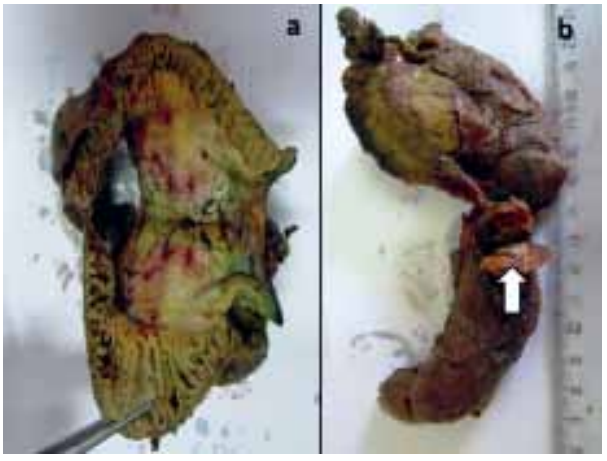


Fig. 2a. Case 1: Macroscopic examination of the surgical specimen showing an ill-defined lesion of the head of the pancreas invading the duodenal wall.
Fig. 2b. Case 1: Macroscopic examination revealing a nodular lesion involving the serosa of the duodenum measuring 1 cm in diameter.



plantation. On admission, physical examination showed mild hepatosplenomegaly and ascites. Laboratory investigations showed thrombocytopenia. Serum ANA and anti-LKM-1 were both negative, but strongly positive for SMA. Metabolic and hepatitis viral markers were negative. Ultrasonography of the liver showed diffuse coarse echotexture. A liver biopsy showed Grade 3 and Stage 4 (METAVIR score) changes, with plasma cell infiltrations, interface hepatitis and rosette formation, consistent with autoimmune hepatitis. Detailed discussions with the transplant team suggested that liver transplantation was indicated. Unfortunately, the patient died perioperatively. During laparotomy, a submucosal nodular lesion involving the ileum was detected. Consequently, segmental ileal resection was performed. Macroscopic

Fig. 3. Case 1: Macroscopic findings. On cut sections, this nodule was yellow in colour with a lobulated appearance reminiscent of pancreatic tissue.

examination of the surgical specimen revealed the presence of a submucosal yellowish lesion measuring 2 cm in diameter. Microscopic examination of the nodule showed heterotopic pancreatic lobules occupying the submucosa under an intact normal ileal mucosa (Fig. 4). The lesion displayed unremarkable pancreatic lobules with acini, ducts and islets of Langerhans (Fig. 5). The final pathological diagnosis was HP of the ileum type I according to Heinrich's classification.

Discussion

The reported frequency of HP during laparotomy is 0.5% and at autopsy is 1.7% [5]. Heterotopic pancreas in the ileum is rare, and when seen, it is usually associated with Meckel's diverticulum. Isolated HP of the ileum is very rare, usually asymptomatic and discovered incidentally during surgery for other conditions as was the case in our patient [6 7]. Some authors have suggested that the presence for the nonspecific symptoms in HP is related to the size and mucosal relation of the pancreatic tissue. Lesions greater than 15 mm and closer to the mucosa are most likely to be symptomatic. Submucosal muscular wall proximity is hypothesized to aggravate bowel dysmotility [6]. Abdominal pain, nausea, vomiting and gastrointestinal bleeding are the most commonly-reported

Fig. 4. Case 2: Heterotopic pancreatic lobules occupying the submucosa under an intact normal ileal mucosa (H&E, original magnification x 10).

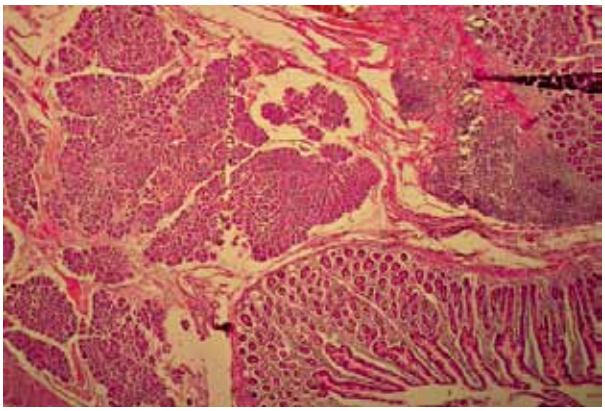
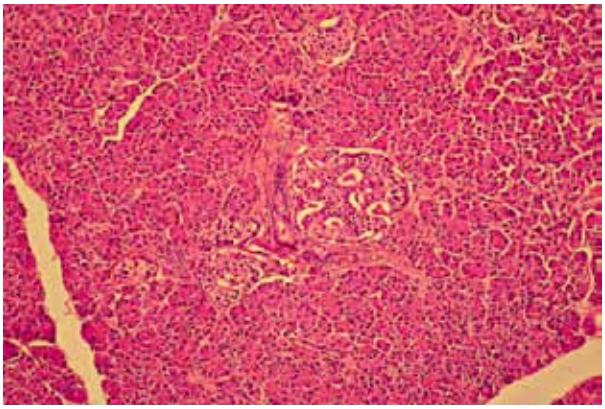


Fig. 5. Case 2: lesion composed of pancreatic acinar structures surrounding ductal structures and scattered islets of Langerhans supporting a diagnosis of pancreatic heterotopia, Heinrich type I (H&E, original magnification x 40).



symptoms [2 7]. Pain associated with HP may be related to the local secretion of hormones and enzymes resulting in tissue inflammation or chemical irritation. Pain may also be related to mechanical obstruction of the intestinal lumen, especially when associated with nausea or vomiting. Rarely, jejunal or ileal lesions may result in intestinal obstruction or intussusception [8-10]. Treatment for symptomatic patients ranges from endoscopic loop excision for superficially located tumours to laparoscopic or other surgical resection [4]. The optimal treatment of histologically-verified asymptomatic HP is unclear. Pancreatic heterotopia grossly resembles normal pancreatic parenchyma as a submucosal nodule, as an intramural mass, or as a nodular lesion involving the serosa [7]. On gross examination, the colour is yellow to yellow-white, and cut sections reveal a lobulated appearance. The size of nodules varies from 0.2 to 4 cm. Histologically, it contains any mixture of tissues that may be found in the normal pancreas. Heterotopic pancreas was classified by Heinrich into 3 types: type I (all elements of the normal pancreatic tissue are present); type II (pancreatic tissue without islet cells); and type III (pancreatic ducts only). Our two cases were considered to be, respectively, type II and type I ectopic pancreas, based on Heinrich's classification. The pathogenesis of HP is unknown. One hypothesis is that during embryonic development, small parts are separated from the pancreas during rotation of the foregut and fusion of the dorsal and ventral portions of the pancreas. These isolated islands of pancreas then continue to grow in the gastrointestinal tract and give rise to ectopic pancreatic tissue. Another theory suggests that pancreatic metaplasia of the endodermal tissue may occur during embryogenesis [7]. Rare complications of HP may include haemorrhage, obstructive jaundice, intraluminal obstruction, intussusceptions, pancreatitis, insulinoma, cyst formation and, rarely, adenocarcinoma or acinar cell carcinoma [11-13].

In summary, two cases of HP of the duodenum and ileum are reported along with pathological findings. The clinical significance of HP is uncertain. There is no correlation between the size, site, or histologic type of HP and the likelihood of malignant transformation. Therefore, observation with periodic endoscopic evaluation is recommended for asymptomatic patients [4].

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Case report

Molecular diagnostics of pulmonary metastasis from cervical cancer

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Key words

Human papillomavirus • Cervical cancer • HPV genotyping • Lung cancer

Summary

High-risk human papillomaviruses (HPV) are largely implicated in the carcinogenesis of cervical carcinomas. Their role in lung carcinomas, however, is still unclear. We describe the case of 44-year-old female chain-smoker with previous HPV-related cervical cancer and a new distant tumour in the lung after many years. The histologic distinction between metastatic squamous cell carcinoma of the cervix and another primary squamous cell tumour of the lung can be difficult and has important clinical implications. The aim of our study was to investigate whether HPV was present in both the patient's cervical cancer and her subsequent primary lung cancer in order to appropriately plan therapy. We tested both the paraffin-embedded tissue of the cervical cancer and the lung cancer for HPV DNA using the Qiagen HPV Sign Genotyping Test, which detected HPV16-DNA in both tumours. The Qiagen HPV Sign Genotyping Test is a reliable method to detect HPV-DNA in tissue and cytological materials, thus making it possible to distinguish metastatic cervical carcinoma from a new primary tumour in different sites.

Introduction

High-risk Human papillomaviruses (HR-HPV) are largely implicated in the pathogenesis of cervical and vaginal carcinomas [1 2]. HR-HPV also play a role in malignant proliferation of the penis, head and neck, and occasionally, the lung [3 6]. In this context, the pathologist needs to distinguish what may be a primary lung tumour from a potential metastasis of squamous cell carcinoma of the cervix uteri. Making this distinction often poses a problem for pathologists, more so as the potential of histologic evaluation is limited in this regard and because clinical discrimination might be difficult [7]. HPV infection is now an established risk factor for the development of squamous cell carcinoma of the cervix [8 9]. Almost 100% of cervical carcinomas are HPV-positive [10]; HPV testing has thus been useful for diagnostic [11] and preventative purposes. Although smoking is the major aetiologic factor of lung cancer [12], the link between HPV and carcinogenesis of lung cancer remains unclear [13].

Case report

A 44-year-old female chain-smoker underwent radical hysterectomy for diagnosis of invasive cervical carcinoma based on F.I.G.O. stage IB1 (no metastasis lymph nodal) in 2004. The patient was later diagnosed with a biopsy-proven invasive squamous cell carcinoma of the left lung. From a morphologic viewpoint, however, the pathologist was not able to determine whether this was a second primary tumour of the lung or a metastasis of the known squamous cell carcinoma of the cervix uteri. As p16 resulted positive in the lung tumour, HPV typing was requested. We therefore performed a Qiagen HPV Sign Genotyping Test on lung cancer biopsy specimen, which was HPV-16 positive (Fig. 1). After that, tissue from the cervical tumour also underwent HPV typing; it also resulted HPV-16 and p16 positive (Fig. 2).

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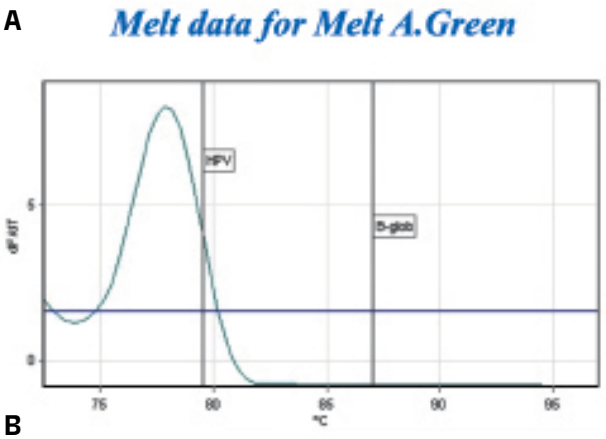
Fig. 1. Results from Rotor Gene and PyroMark Q24 of lung cancer biopsy.

A: HPV-positive lung cancer biopsy based on Rotor-Gene instrument, the HPV and β -globin melt peaks can be distinguished from each other thanks to the different melting temperature of the amplified DNA fragments **B**: HPV 16 lung cancer biopsy through PyroMark Q24 instrument based on their alignment with sequences contained in the HPV library with IdentiFire sw.

A



B

Hit 1: Human papillomavirus type 16

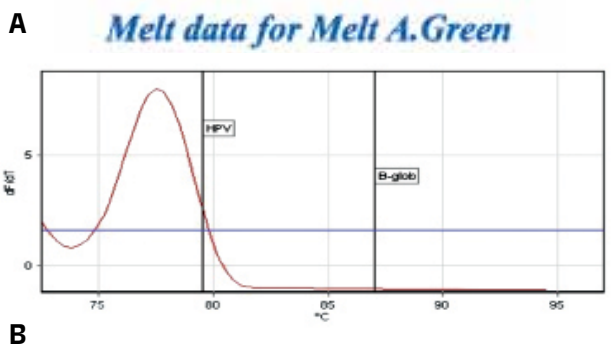
Score: 100; Identities: 30/30 (100%); Gaps: 0/30 (0%)

```
Query 1    AACTACATATAAAAATACTAACTTTAAGGA 30
           | || ||| || ||| || || ||| || || ||||| || | |
Library 1  AACTACATATAAAAATACTAACTTTAAGGA 30
```

Fig. 2. Results a Rotor Gene and PyroMark Q24 of cervical cancer.

A: HPV-positive cervical cancer surgical tissue based on Rotor-Gene instrument, the HPV and β -globin melt peaks can be distinguished from each other thanks to the different melting temperature of the amplified DNA fragments. **B**: HPV 16 cervical cancer surgical tissue through PyroMark Q24 instrument, based on their alignment with sequences contained in the HPV library with IdentiFire sw

A



B

Hit 1: Human papillomavirus type 16

Score: 100; Identities: 30/30 (100%); Gaps: 0/30 (0%)

```
Query 1    ACGCAGTACAAATATGTCATTATGTGCTGC 30
           || || |  | ||| || ||| || || || |||| || | |||
Library 1  ACGCAGTACAAATATGTCATTATGTGCTGC 30
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Methods

We used the Qiagen HPV Sign Genotyping Test (Q24) to examine a formalin-fixed, paraffin-embedded lung tumour biopsy (Fig. 3) and a specimen of cervical tumour (Figure 4) for the presence of HPV. This test allows HPV virus detection and genotyping using Rotor-Gene and PyroMark Q24 instruments. After amplification of DNA extracted from biopsy specimen on Rotor-Gene Q, screening and genotyping were performed through melt curve analysis and Pyrosequencing, respectively. Rotor-Gene Q amplifies nucleic acids using an innovative rotor system. The HPV and β-globin melt peaks can be distinguished from each other thanks to the different melting temperatures of the amplified DNA fragments.

The pyrosequencing on which PyroMark Q24 is based is a sequencing technology that uses synthesis. The sequencing primers HPV 1 primer PQ, HPV 2 primer PQ, HPV 3 primer PQ, and HPV 4 primer PQ allow the synthesis of genotype-specific sequences of 30 bases. These sequences have a high discriminatory power, making it possible to identify the HPV genotype present in the sample based on its alignment with sequences contained in the HPV library with IdentiFire software. There are over 100 genotypes of HPV identified and classified according to genomic homology.

Fig. 3. A: Haematoxylin-eosin lung cancer biopsy. **B**: P16-positive lung cancer biopsy (immunostaining). The inset at the upper right corner shows p16-positive lung cancer cells at high power.

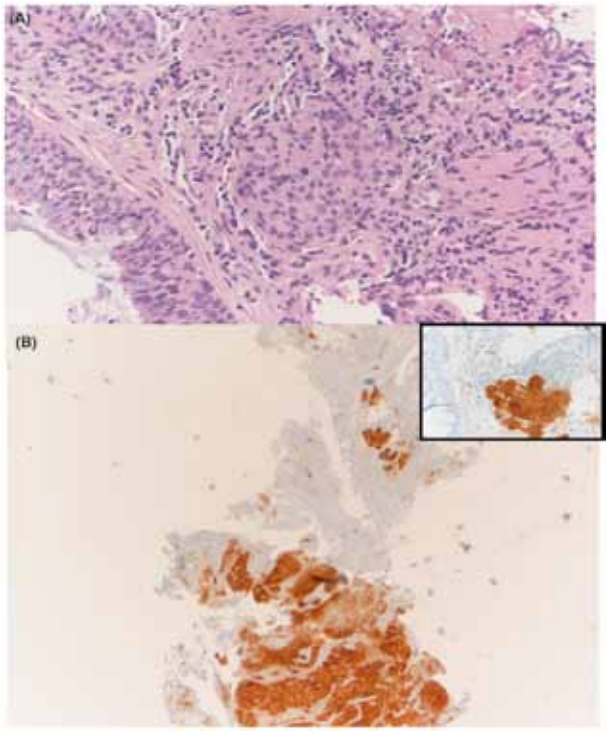
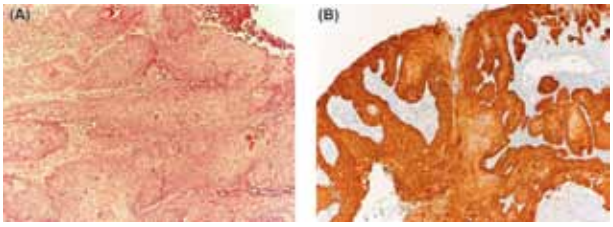


Fig. 4. A: Haematoxylin-eosin cervical squamous cell carcinoma is shown. **B**: P16-positive cervical cancer is shown (immunostaining).



Discussion

This study employed PCR to detect HPV DNA in paraffin-embedded tissue of cervical carcinoma and in a metastasis from the same patient. In general, PCR offers many possibilities for all types of retrospective studies on archival material. More specifically, the relationship between HPV in cervical carcinomas and their metastasis supports the suggested role of HPV in the pathogenesis of cervical cancer. In some isolated cases, HPV DNA has been detected in metastatic tumours. Because PCR is sensitive, it must be performed carefully. Indeed, its sensitivity can be a disadvantage if no precautions are taken to avoid detection of contamination with cloned HPV plasmids or PCR products themselves. In our laboratory, we introduced breaking down the PCR technique into different steps. Sample preparation, production of reaction mixtures and primers, and amplification and detection are performed at different locations. An important feature of PCR on paraffin-embedded tissue is that the reaction is not inhibited by the fixatives used. As HPV-16 was detected in both tumours in our patient, the lung tumour was confirmed as cervical cancer metastasis, and thus not due to the fact that the patient was a chain-smoker. In the literature, cervical cancer positive to HPV16 more frequently produces metastasis than do other HPV genotypes. As the Qiagen HPV Sign Genotyping Test is extremely sensitive, it can be employed to detect HPV infection in not only cervix uteri, but also in other organs where the presence of the virus has important clinical implications.

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Case report

Pure uterine lipoma

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Key words

Uterine Lipoma • Lipoma

Summary

Pure uterine lipoma is a very rare benign mesenchymal neoplasm, and only a few cases have been reported in the literature. This is in contrast to leiomyoma, which is not only the most common neoplasm of the uterus but also one of the most common tumours in women, estimated to occur in 20-40% of women beyond the age of 30 years (AFIP) and more frequently affect postmenopausal women. We report the case of a 70-year-old woman who presented with pelvic pain and postmenopausal uterine bleeding. Pure uterine lipoma was diagnosed preoperatively by CT scan with and without contrast and confirmed postoperatively by pathological examination. Clinical and histological diagnosis of pure uterine lipoma with immunohistochemical findings are described,and the efficacy of CT in diagnosing this tumour is discussed.

Introduction

Pure lipomas are rare benign neoplasms of the uterus; only a few more than 200 cases have been reported in literature, with an incidence that varies from 0.3 to 0.12% [1-3]. Clinical symptoms and physical signs are similar to those of uterine leiomyoma. These tumours usually occur in postmenopausal women from 50 to 70 years of age [4-6], arise in the uterine corpus and are generally intramural [7 8].

Case report

A 70-year-old woman was admitted to the ward of Obstetrics and Gynaecology with a complaint of discomfort caused by pelvic pain and abnormal uterine bleeding lasted for an extended period of time.
An ultrasound scan showed increased uterine volume, multiple nodular lesions and a thin polipous endometrium. On post-contrast computed tomography (CT), a homogeneous, adipose density, well circumscribed and partially septated tumour with scarce solid component was not enhanced, and the tumour was contiguous with the left uterine adnexa and therefore interpreted as a benign neoplasm of left ovary (Fig. 1). The margins were well-defined and no lymphoadenomegaly was noted. Initial nodular goitre was also present.
In view of the CT result, the surgeons opted for surgical intervention and performed a total abdominal hysterectomy and bilateral salpingo-oophorectomy.
On gross examination, the uterus weighed 670 gm. The uterus body was deformed by two soft-yellow, well circumscribed intramural masses, the largest one measuring 8 cm.
Histologically, the tumours were composed of mature adipose cells separated by thin fibrovascular septa without any evidence of malignancy (Fig. 2). Endometrium with atrophic glandular epithelium (Fig. 3).
An immunohistochemical study was performed with actin (Monoclonal HHF35 Novocastra, prediluted), desmin (polyclonal Novocastra, prediluted), vimentin (Monoclonal V 9 Novocastra, prediluted), CD68 (Monoclonal KP-1 Novocastra, prediluted), S-100 protein (polyclonal Novocastra), oestrogen receptor (ER) (Monoclonal 6F-11 Novocastra, prediluted), progesterone receptor (PR) (Monoclonal 1A6 Novocastra, prediluted), CD34 and Ki-67. Muscular cells and blood vessels were used as internal positive controls.
Smooth SMA muscle cells of the myometrium were reactive to actin and desmin but not to the tumour cells (Figs. 4, 5). S-100 protein (Fig. 6) and vimentin (Fig. 7) were found in lipomatous cells while CD68, oestrogen receptor (ER), progesterone receptor (PR) were not present (Figs. 8, 9); focal actin and desmin were found in the septa,

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Fig. 1. The post-contrast CT-scan of pelvis showes a well circumscribed, homogeneus and adipose density mass in the uterine wall.

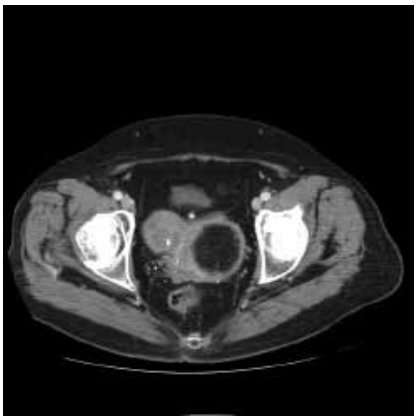


Fig. 2. The tumor is composed of mature adipose cells (H§E 10X).

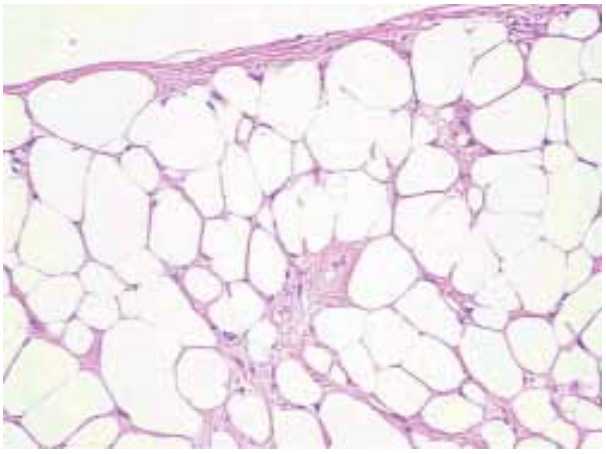


Fig. 3. Endometrium with atrophic glands (H.E 10X).

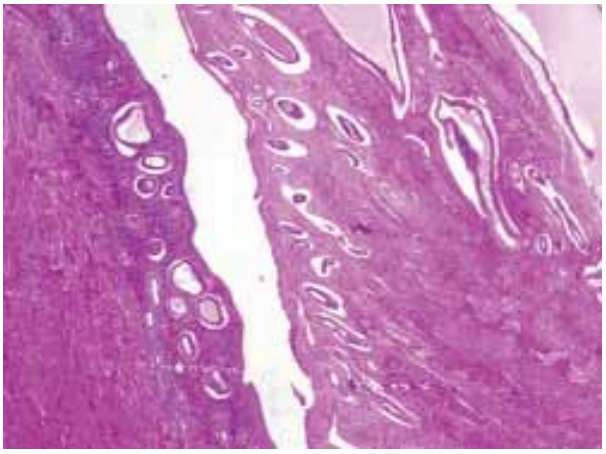


Fig. 4. Actin (SMA) staining: the tumour cells are negative.

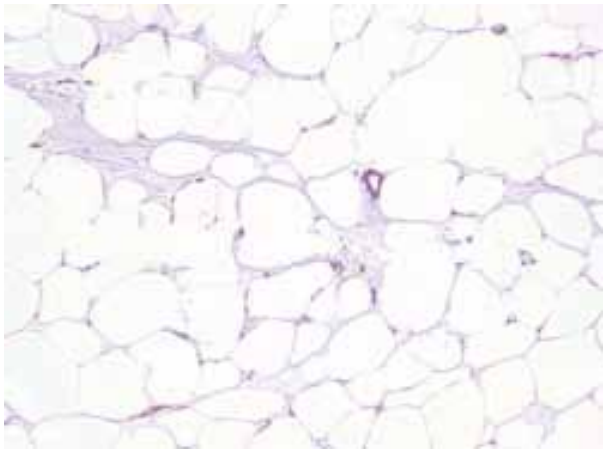


Fig. 5. Desmin staining: the tumour cells are negative.

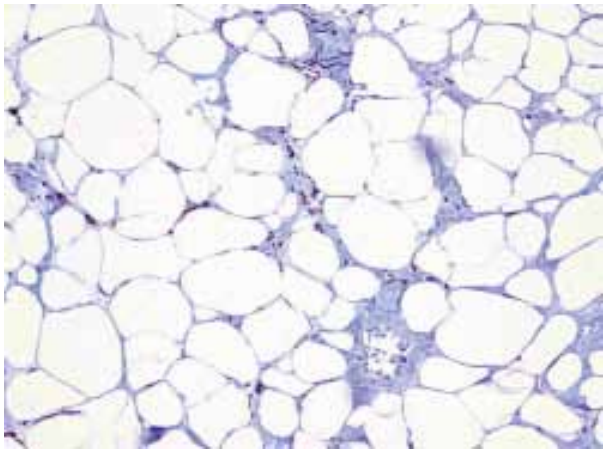


Fig. 6. S100 Protein: The tumour cells are positive.

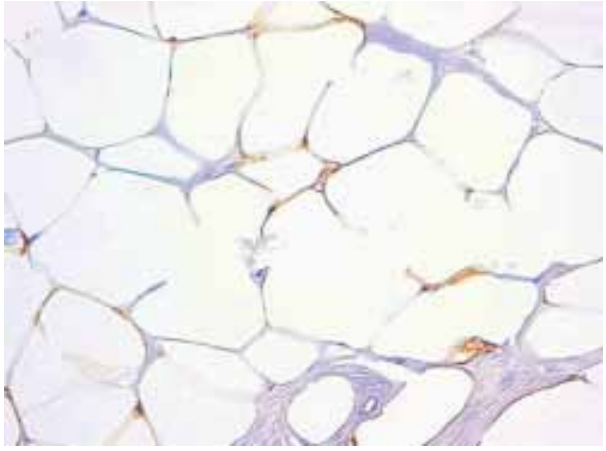


Fig. 7. Vimentin staining. The tumour cells are positive.

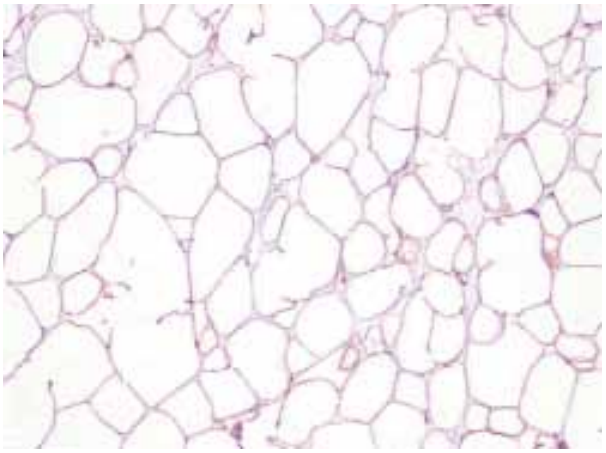


Fig. 8. Oestrogen receptor (ER) is not presente.

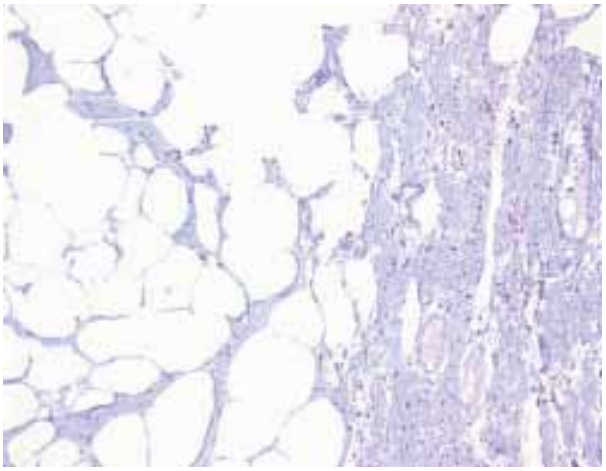
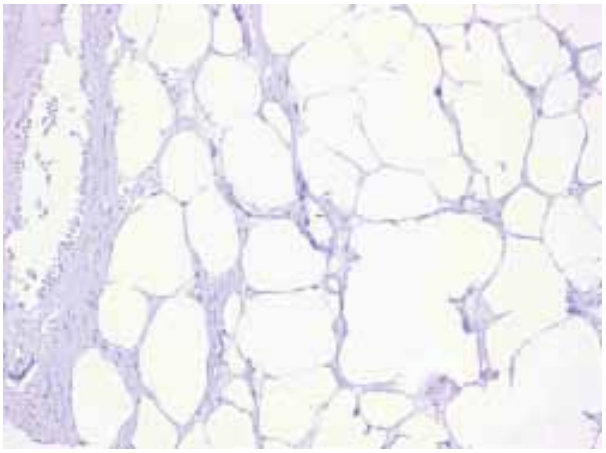


Fig. 9. Progesteron receptor (PR) is not present.



and CD34 was present in a few vascular structures of the tumour (Fig. 10). No pronounced proliferation rate was noted by Ki-67 (Fig. 11).

Discussion

Lipomatous tumors of the uterus are rare, benign neoplasms that usually occur mixed with other mesenchymal neoplasms, often with leiomyomas (lipoleiomiomas). Uterine lipomas and pure homologous mesenchymal tumour usually occur in postmenopausal women and, if of large dimension, manifest with symptoms similar to those of uterine leiomyomas as pelvic pain and uterine abnormal bleeding. On ultrasound, lipomas show a high-intensity signal consistent with fatty tissue surrounded by a hypoechoic area. CT can provide more specific

Fig. 10. CD34 staining showes a few vascular structures of the tumour.

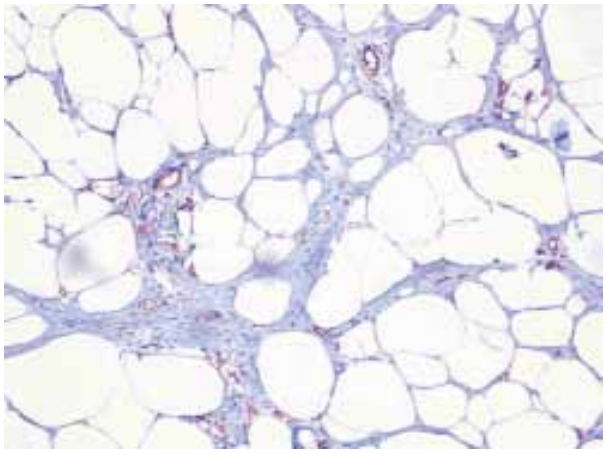
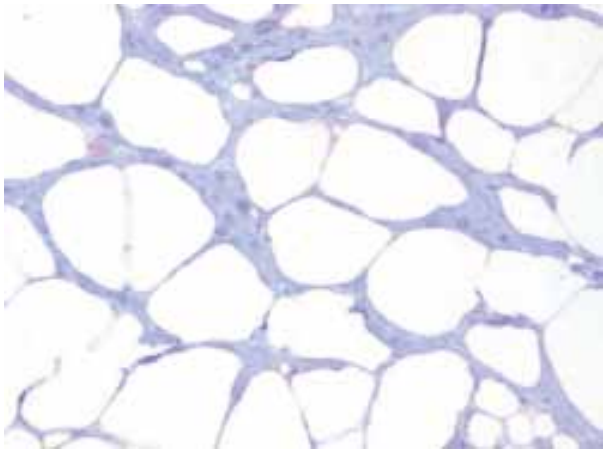


Fig. 11. Growth fraction. Staining for Ki67/MIB1.



findings by showing well circumscribed mass with the density of fat. However, if the tumour is large and the continuity of the tumour with the cervix is unclear, it is very difficult to differentiate a uterine tumour from an ovarian tumour using CT, as we observed.
In our case, a tumour with density of fatty tissue of left ovary was suspected following CT. The differentiation between a uterine tumour and an ovarian tumour was impossible because of the tumour bulk. Some authors consider MRI to be the best tool for pre-operative diagnosing of pelvic fatty tumours [13].

Conclusions

Our study confirms that pure lipomatous tumors of the uterus are very rare, benign neoplasms. Their histogenesis is still unclear and controversial. There are several hypotheses in this regard: misplaced embryonic mesodermal remains with a potential for lipoblast differentiation [9], transformation of a totipotent mesenchymal cell [10] or degenerative-metaplastic processes of smooth muscle or stromal cells [11], or proliferation of perivascular fat cells accompanying the blood vessels in the uterus [12].

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Linee Guida

Il carcinoma del pancreas esocrino: il referto istologico

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Parole chiave

Adenocarcinoma duttale • Referto GIPAD • Neoplasia intraduttale • Pancreas

Riassunto

Il gruppo Gruppo Italiano Patologi Apparato Digerente (GIPAD) ha nominato un comitato per formulare suggerimenti relativi alla refertazione anatomo-patologica del cancro del pancreas. Il comitato, formato da patologi con specifica esperienza su questo tipo di neoplasia, ha scritto i suggerimenti che sono stati rivisti e approvati dai responsabili del gruppo GIPAD. I suggerimenti sono stati suddivisi in diversi sottocapitoli che comprendono: la descrizione macroscopica, il trattamento del pezzo macroscopico, la diagnosi istopatologica, l'immunoistochimica, le indagini molecolari e la lista di controllo. Lo scopo di questi suggerimenti è quello di fornire ai clinici un referto pienamente informativo.

Introduzione

L'adenocarcinoma duttale (ACD) del pancreas è una delle neoplasie più gravate da un'elevata mortalità. Poche terapie sono efficaci e fino a tempi relativamente recenti poco si è conosciuto sulla patogenesi di questa malattia. In questi ultimi venti anni si sono registrati vari progressi nel campo della patologia pancreatica che permettono una migliore comprensione dei meccanismi patologici coinvolti nel cancro pancreatico (CP) e un migliore trattamento dei pazienti. Questi comprendono l'identificazione di alcuni eventi molecolari chiave nella patogenesi dell'ACD [1]. Si presume che lo sviluppo dell'ACD sia preceduto da lesioni proliferative intraduttali quali la neoplasia pancreatica intraepiteliale (PanIN), lesioni che sono oggi ben caratterizzate [2]. Sono state identificate varianti del CP con precise caratterizzazioni genetiche e cliniche [3]. I sottotipi e il comportamento biologico delle neopalsie cistiche del pancreas, compresa la neopalsia cistica mucinosa e la neoplasia intraduttale papillare mucinosa sono ora meglio definite e questo è importante perché le neoplasie cistiche possono essere diagnosticate e curate prima che si sviluppi un cancro invasivo [4]. Infine, è ora evidente che il CP si aggrega in alcune famiglie e che alcuni geni responsabili delle aggregazioni familiari del CP sono stati identificati [5]. Noi abbiamo considerato tutti i progressi ottenuti nelle conoscenze della patologia pancreatica, riportati sopra, con l'intento di fornire un referto del CP pienamente informativo per il clinico.

Epidemiologia

L'ACD e le sue varianti costituiscono la neoplasia più frequente del pancreas, rappresentando l'85-90% di tutte le neopalsie pancreatiche [6]. L'incidenza del CP raggiunge i tassi più elevati (circa 12 per 100000 maschi e 10 per 100000 femmine) tra gli afro-americani e le popolazioni indigene dell'Oceania, mentre i tassi più bassi (< 2 per 100000) sono registrati nell'Africa centrale e nel sud-est dell'Asia [1 7]. Nell'anno 2000 sono stati riportati, in tutto il mondo, 217000 nuovi casi di CP e le morti per CP sono state 213000; in Europa i nuovi pazienti sono stati 60139 (il 10,4% di tutti i cancri dell'apparato digerente), con 64801 morti [8]. In Italia, nel periodo tra il 1998 e il 2002, il CP è stato all'11°-10° posto tra i cancri più frequenti nei maschi (2,2% di tutti i cancri) e delle femmine (2,8% di tutti i cancri). Ha rappresentato la 7ª causa più frequente di morte per cancro (4,6% di tutte le morti per cancro) tra i maschi e la 6° (6,6%) tra le femmine. È stato stimato che ogni anno in Italia vengono diagno-

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sticati 4388 nuovi casi di CP tra i maschi e 4214 CP tra le femmine. La mortalità è stata, nel 2002, di 4069 per i maschi e di 4280 tra le femmine. L'incidenza non è variata in tutta l'Italia e il rapporto tra le aree con l'incidenza più elevata e quelle con l'incidenza più bassa è stata di circa 2 [9].
La sopravvivenza mediana dei pazienti con CP metastatico non sottoposti a terapia attiva è di 3-5 mesi e di 6-10 mesi nel caso di malattia localmente avanzata; essa sale a circa 11-15 mesi col trattamento chirurgico resettivo [10].
A causa della comparsa tardiva dei sintomi e del comportamento aggressivo del tumore, solo una minoranza di pazienti può essere sottoposta a chirurgia radicale, potenzialemente curativa. I progressi maggiori negli ultimi 10 anni hanno compreso miglioramenti nella mortalità e morbidità operatoria tramite lo sviluppo di centri di riferimento multidisciplinari e miglioramenti della sopravvivenza utilizzando la chemioterapia sistemica [10].

Aspetti clinici [11]

La diagnosi clinica di CP si basa su sintomi comuni che comprendono:
- dolore alla parte alta dell'addome che si irradia caratteristicamente al dorso;
- ittero indolente quando il cancro della testa ostruisce il coledoco;
- perdita dell'appetito e/o nausea e vomito;
- grave e rapida perdita di peso.

Il segno di Trousseau da ipercoagulabilità, con formazione di trombosi spontanea nei vasi portali, nelle vene profonde delle estremità o nelle vene superficiali in qualsiasi sede corporea, è a volte associato al CP.
I test funzionali epatici possono mostrare una combinazione di risultati suggestiva di un'ostruzione biliare con elevazione dei livelli della bilirubina coniugata, della γ-glutamil-transpeptidasi e della fosfatasi alcalina. Il CA-19-9 (antigene carboidrato 19.9) è il marcatore più comunemente utilizzato per il CP e ha una sensibilità del 70-90% e una specificità del 90% ed è migliore di altri marcatori quali il CEA, il CA-50 e il DUPAN-2 [11]. Le tecniche diagnostiche disponibili, compresa l'ultrasonografia transaddominale (TUS), la tomografia computerizzata (TC), la risonanza magnetica (RM) e l'ecoendoscopia (EUS) sono superiori ad altri test di screening non chirurgici nell'individuazione di lesioni pancreatiche.
In analisi recenti sull'accuratezza diagnostica di varie tecniche, la TUS ha dimostrato un'accuratezza diagnostica del 75%, la TC con contrasto del 97% e la RM circa la stessa percentuale della TC [11].

Anatomia patologica [1 4 6]

Diagnosi anatomo-patologica preoperatoria

La conferma anatomo-patologica della diagnosi di neoplasia prospettata dalle immagini e dai marcatori tumorali è richiesta per evitare laparotomie diagnostiche non necessarie e per classificare precisamente il tipo di tumore prima di una resezione chirurgica maggiore del pancreas, di cui sono noti i rischi di mortalità e morbilità.
La valutazione anatomo-patologica può basarsi sull'esame citologico di un agoaspirato con ago sottile (FNA) e/o di uno spazzolato endoscopico o su biopsie tessutali. La citologia da FNA guidata dalla TUS e dalla TC ha una sensibilità del 69% e una specificità del 100% [10].
La sensibilità e la specificità dell'EUS con FNA sono rispettivamente del > 90% e del 100%, ma richiedono un gruppo di esperti con la presenza di un citologo che valuti l'adeguatezza del materiale citologico [12].
La FNA intraoperatoria, guidata dall'ultrasonografia, è meno traumatica, più sicura e più accurata in termini diagnostici, delle biopsie intraoperatorie, sia incisionali che da ago grosso. Con la FNA i rischi diretti di emorragia, pancreatite e formazione di ascessi possono essere evitati e si possono eseguire aspirazioni multiple da aree diverse, senza correre rischi. La FNA intraoperatoria consente una differenziazione precisa tra una pancreatite cronica e un CP nel 95-100% dei casi [13]. Di conseguenza, la FNA è considerata la tecnica diagnostica più sicura e più accurata per la valutazione intraoperatoria di una massa pancreatica, specialmente se questa è situata nella parete profonda della ghiandola.
In molti casi, il materiale aspirato permette non solo di riconoscere le cellule maligne, ma anche di distinguere un adenocarcinoma da una neoplasia neuroendocrina e, con l'aiuto dell'immunoistochimica, di identificare i diversi tipi di tumori esocrini ed endocrini (Tab. I).
Le neoplasie pancreatiche che interessano il coledoco possono essere identificate su preparati citologici da spazzolamento. Questa tecnica ha una sufficiente sensibilità e specificità diagnostica [14], anche se i risultati falsi positivi e falsi negativi sono più alti se confrontati con quelli della FNA [15].
Tutti i metodi bioptici descritti permettono una diagnosi istopatologica (Tab. III).
Un'agobiopsia può essere ottenuta preoperatoriamente con aghi di calibro 18-20 gauge guidati da TC, TUS, EUS o intraoperatoriamente con un ago di Silverman o di calibro simile sotto il controllo visivo diretto del chirurgo. Biopsie a cuneo possono essere ottenute intraoperatoriamente e sono adeguate per una diagnosi su sezioni criostatiche.
Biopse mirate possono essere ottenute preoperatoriamente in laparoscopia, anche con l'ausilio dell'ultrasonografia endoscopica: una tecnica che è utilizzata anche per definire l'estensione della malattia e per monitorare i risultati del trattamento [16].

Tab. I. Aspetti diagnostici di malignità in FNA.

Aggregati piccoli o grandi di cellule epiteliali strettamente coese
Nuclei ingranditi, irregolari, con nucleoli prominenti e perdita della polarità nucleare
Citoplasma in genere scarso
Mitosi occasionali

Tab. II. Aspetti diagnostici di malignità in citologia da spazzolamento delle vie biliari.

Cellularità elevata
Sottofondo di necrosi
Compressione e fusione nucleare

Tab. III. Aspetti diagnostici di malignità in adenocarcinomi duttali indipendentemente dal tipo di biopsia,

Aumento del numero di strutture ghiandolari
Distribuzione disordinata dei dotti
Lumi ghiandolari incompleti
Materiale necrotico nei lumi ghiandolari
Variazione della dimensione dei nuclei tra le cellule duttali
Invasione perineurale

Esame macroscopico del pezzo operatorio, trattamento e descrizione

Informazione clinica richiesta

Informazione clinica che deve essere fornita al patologo per l'esame dei campioni rimossi dal paziente con un carcinoma del pancreas esocrino:

Dati di identificazione
- Nome
- Data di nascita
- Sesso

Medico responsabile
Data dell'intervento
Altre informazioni cliniche
- Storia clinica
- Ittero
- Pancreatite
- Diabete mellito
- Cancri familiari o ereditari
- Altro

Immagini e referti endoscopici
Diagnosi clinica
Procedura specifica (FNA, spazzolamento, agobiopsia, biopsia a cuneo, pancreasectomia parziale, resezione secondo Whipple)
Reperti intraoperatori
Sede anatomica del campione

Dissezione e descrizione del pezzo di resezione pancreatico

Tipo di campione e organi presenti nel pezzo

Il tipo di campione deve essere indicato, ad esempio: duodenocefalopancreasectomia (DCP) standard secondo Whipple, DCP con preservazione del piloro, pancreasectomia totale, pancreasectomia distale (PD) o sinistra, resezione segmentaria del pancreas.

La DCP secondo Whipple consiste nella resezione della testa del pancreas con il duodeno e la parte distale dello stomaco, la colecisti e il digiuno prossimale con rimozione in blocco dei linfonodi regionali. La parte distale dello stomaco non è resecata nella DCP con preservazione del piloro (Figg. 1, 2). La pancreasectomia totale comprende anche il corpo e la coda del pancreas con

Fig. 1. Margini di resezione in un pezzo operatorio pancreatico da DCP con preservazione del piloro. a = margine duodenale prossimale; b = margine del dotto cistico; c = margine del coledoco; d = margine pancreatico (con il dotto principale); e = margine duodenale distale; f = margine anteriore; g = margine mediale (margine della doccia mesenterica); h = margine posteriore.

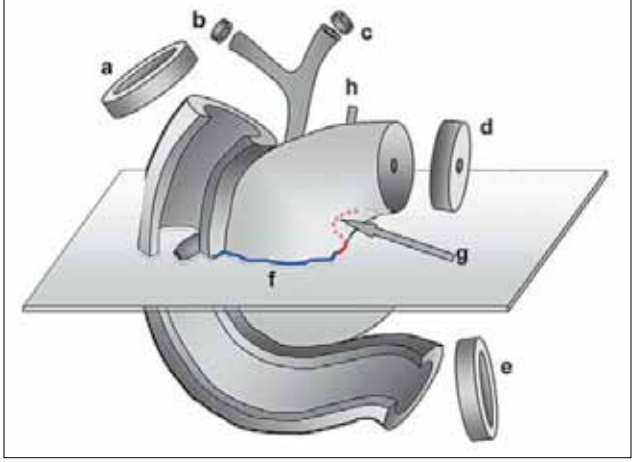


Fig. 2. Margini di resezione in un pezzo operatorio pancreatico da DCP con preservazione del piloro. a = margine anteriore; b = margine mediale (doccia mesenterica); c = margine posteriore; d = duodeno; e = papilla di Vater.

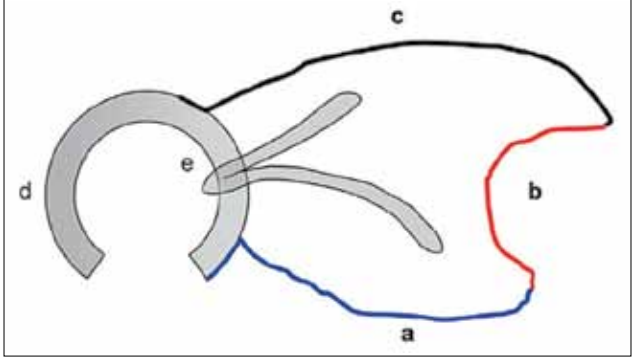
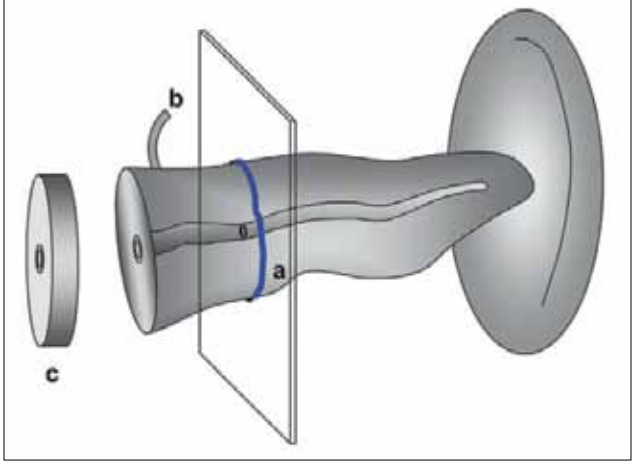


Fig. 3. Margini di resezione di un pezzo operatorio pancreatico da pancreasectomia distale (sezione trasversale). a = margine anteriore; b = margine posteriore; c = parenchima pancreatico.



o senza la milza e/o lo stomaco. Nella pancreasectomia distale (o sinistra) sono presenti il corpo e la coda del pancreas con o senza la milza (Fig. 3).

Trattamento del pezzo ed esame macroscopico

I campioni da pancreasectomia vanno esaminati a fresco, prima della fissazione (questo è meno importante per le resezioni distali), possibilmente in stretta collaborazione con i chirurghi.

Il coledoco e il dotto pancreatico principale dovrebbero essere specillati e l'intero pezzo sezionato lungo gli specilli. La sede di origine della neoplasia deve essere identificata con precisione, in modo da escludere un carcinoma ampollare visto che quest'ultimo si associa a una prognosi decisamente migliore [17]. Le neoplasie che interessano la testa pancreatica dovrebbero essere identificate come segue:

1. tumore pancreatico: una neoplasia localizzata nella testa pancreatica;
2. tumore ampollare: una neoplasia centrata nell'ampolla;
3. tumore periampollare: una neoplasia in stadio avanzato il cui preciso punto di origine non è identificabile;
4. tumore del coledoco terminale: una neoplasia localizzata nel terzo inferiore del coledoco.

La sede del tumore dovrebbe quindi essere riportata in relazione all'ampolla e al coledoco e la distanza da entrambi dovrebbe essere indicata.

L'invasione delle strutture adiacenti (duodeno, coledoco, tessuti molli peripancreatici) deve essere riportata e la grandezza del tumore è un fattore prognostico indipendente nella maggior parte degli studi [18]. Aspetti quali la formazione di cisti, la crescita tumorale intraduttale e la presenza di muco nei dotti dilatati dovrebbero essere descritti, essendo questi quadri caratteristici di tumori pancreatici generalmente associati ad una buona prognosi, compresa la neoplasia mucinosa cistica [19] e la neoplasia intraduttale papillare mucinosa [20].

La pancreasectomia distale (sinistra) è il trattamento di scelta per i tumori del corpo e della coda. La dimensione del tumore e la sua distanza dal margine di resezione parenchimale dovrebbe essere misurata e qualsiasi invasione del tessuto peripancreatico annotata. Gli aspetti macroscopici e in particolare la presenza di cisti o la forma solida con contorni lisci devono essere riportati, essendo essi indicativi di specifici tipi tumorali quali la neoplasia cistica mucinosa e la neoplasia solida pseudopaillare.

Per i tumori cistici devono essere riportati: il contenuto cistico (mucinoso, sieroso, ematico, necrotico), l'aspetto uni- o multi-loculare, la superficie interna (liscia o papillare), la presenza di noduli murali e la comunicazione con i dotti pancreatici maggiori. In questi casi il campionamento deve essere esteso, in modo da individuare possibili focolai infiltrativi.

Margini di resezione

La completezza della resezione deve essere accertata con l'esame macroscopico e confermata con l'esame istologico [21-23].

I margini di resezione comprendono il margine coledocico, il margine trasversale pancreatico (con il dotto principale) e il margine trasversale duodenale (Fig. 1). Sia il margine di resezione coledocico che quello pancreatico dovrebbero essere valutati intraoperatoriamente su sezioni al criostato.

Molto importante è il margine di resezione circonferenziale. È definito come margine anteriore, mediale e posteriore, sul tessuto adiposo peripancreatico dietro la testa del pancreas; in particolare il margine mediale è situato dorsalmente e lateralmente all'arteria mesenterica superiore, ha la forma di un solco poco profondo("doccia mesenterica") e deve essere esaminato con attenzione per trovare un'eventuale infiltrazione neoplastica (Figs. 1, 2). È importante anche considerare il rivestimento sieroso della superficie pancreatica anteriore, in modo da escludere una diffusione sierosa neoplastica.

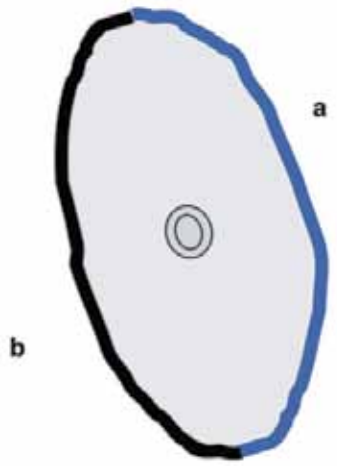
Nelle pancreasectomie sinistre vanno considerati il margine circonferenziale sul tessuto adiposo peripancreatico e il margine trasversale sul corpo (Figs. 3, 4). Il margine di resezione trasversale dovrebbe essere valutato intraoperatoriamente su sezioni criostatiche.

Tutti i margini di resezione dovrebbero essere marcati con inchiostro di china e dovrebbero essere campionati. Il tessuto di ogni margine di resezione dovrebbe essere sezionato perpendicolarmente alla superficie e sezioni successive, numerate, dovrebbero essere sottoposte all'esame istologico. I campioni da includere vengono presi dal parenchima pancreatico, sezionati ad intervalli di 2 mm (come fette di pane) con una lama affilata, per valutare i rapporti tra il tumore e i margini di resezione, il duodeno, l'ampolla di Vater, il parenchima privo di neoplasia e gli altri organi [24].

Esame dei linfonodi

I linfonodi asportati devono essere classificati e numerati, per una valutazione routinaria, secondo il sistema TNM [1 25]. I linfonodi regionali del pancreas possono essere raggrupati in: pancreatico-duodenali anteriori,

Fig. 4. Margini di resezione di un pezzo operatorio da pancreasectomia distale (sezione trasversale). a = margine anteriore; b = margine posteriore.



pancreatico-duodenali posteriori, inferiori (compresi quelli che circondano i vasi mesenterici superiori), coledocici, infrapilorici e superiori (per i tumori della testa del pancreas) [26]. Il campionamento dei linfonodi deve essere accurato dato che lo stato linfonodale è un fattore prognostico importante [27 28]. Nella nostra esperienza è indispensabile un campionamento integrale del tessuto adiposo peripancreatico per poter eseguire un'analisi completa di tutti i linfonodi, poiché essi sono di frequente molto piccoli e non sono facilmente identificabili nel tessuto fibroadiposo peripancreatico: nei campioni di DCP, i linfonodi sono spesso disposti nel solco creato dalla giunzione del pancreas e dalla parete intestinale; nelle pancreasectomie distali essi sono più spesso collocati nel tessuto adiposo perivascolare.

Tab. IV. Classificazione WHO dei tumori epiteliali maligni del pancreas*.

Descrizione	ICDO
Adenocarcinoma duttale	8500/3
Carcinoma adenosquamoso	8560/3
Carcinoma colloide (carcinoma mucinoso non cistico)	8480/3
Carcinoma epatoide	8576/3
Carcinoma midollare	8510/3
Carcinoma a cellule ad anello con castone	8490/3
Carcinoma indifferenziato	8020/3
Carcinoma indifferenziato con cellule giganti osteoclasto-simili	8035/3
Carcinoma a cellule acinose	8550/3
Cistoadenocarcinoma a cellule acinose	8551/3
Neoplasia intraduttale papillare mucinosa con carcinoma invasivo associato	8453/3
Carcinoma misto acinoso-duttale	8552/3
Carcinoma misto acinoso-neuroendocrino	8154/3
Carcinoma misto acinoso-neuroendocrino-duttale	8154/3
Carcinoma misto duttale-neuroendocrino	8154/3
Neoplasia mucinosa cistica con carcinoma invasivo associato	8470/3
Pancreatoblastoma	8971/3
Cistoadenocarcinoma sieroso	8441/3
Neoplasia solida pseudopapillare	8452/3

*con l'esclusione delle neoplasie neuroendocrine pure

Tutti i linfonodi devono essere sottoposti all'esame istologico separatamente e i linfonodi di diametro maggiore di 1 cm devono essere tagliati a metà.

Esame microscopico

Tutti i tessuti campionati devono essere inclusi in paraffina e colorati con ematossilina-eosina. Una sezione del campione tumorale deve essere colorata con Alcian blu-PAS; colorazioni opzionali comprendono le colorazioni per le fibre elastiche per i vasi e vari immunocolorazioni (CK7, 8, 18, 19, MUC1, MUC3, MUC4, MUC5AC, CEA, CA19-9, CA125, DUPAN2, mesotelina, antigene delle cellule staminali prostatiche (PSCA), claudina4, DPC4, p16, p53).

Tipizzazione istologica tumorale e graduazione

La tipizzazione deve essere fatta secondo i criteri universalmente accettati della WHO [29] (Tab. IV).

Sebbene più del 90% dei carcinomi siano adenocarcinomi duttali (comprese le varianti), altre neoplasie maligne come i carcinomi acinari e i carcinomi neuroendocrini devono essere presi in considerazione. Essi vanno distinti dai tumori metastatici o dalle neopalsie maligne mesenchimali.

Come si è accennato prima, la diagnosi differenziale più importante è quella con il carcinoma ampollare. Un'origine ampollare può essere stabilita inequivocabilmente nel caso di lesioni piccole, applicando criteri topografici stretti, nel corso dell'esame macroscopico e dell'esame istologico. La presenza di lesioni "preinvasive" (adenomatose) nelle strutture anatomiche dell'ampolla e di un adenocarcinoma di tipo intestinale sono di aiuto per la distinzione dall'ACD [17 30].

È soprattutto importante identificare le neoplasie mucinose cistiche e le neoplasie intraduttali papillari mucinose perché esse si associano a una prognosi decisamente migliore.

La variante "cistica" dell'ACD, che è dovuta o a alterazioni degenerative o a dilatazioni del sistema duttale può mimare le due neoplasie menzionate prima.

Per l'ACD il grado è un fattore prognostico essenziale e indipendente e deve essere registrato secondo i criteri WHO [1] (Tab. V).

Invasione locale

La stadiazione TNM [26 29] richiede di stabilire se il carcinoma pancreatico abbia invaso o no il duodeno, l'ampolla di Vater, il coledoco o i tessuti peripancreatici (T3) o

Tab. V. Grado istologico dell'adenocarcinoma pancreatico duttale.

Grado	Differenziazione ghiandolare	Produzione di mucine	Mitosi (per 10 HPF)	Aspetti nucleari
1	Ben differenziata	Abbondante	≤ 5	Scarso polimorfismo, disposizione polare
2	Strutture simil-duttali e ghiandolari tubulari moderatamente differenziate	Irregolare	6-10	Polimorfismo moderato
3	Ghiandole scarsamente differenziate e strutture mucoepidermoidi abortive e pleomorfe	Abortiva	> 10	Marcato polimorfismo e dimensioni aumentate

abbia invaso lo stomaco, la milza, il colon o i grossi vasi adiacenti. L'invasione dei tessuti molli peripancreatici è riscontrata fino al 90% dei casi [21] e indica una prognosi sfavorevole.

Ampiezza della resezione

L'interessamento neoplastico dei margini di resezione standard implica una recidiva locale frequente e si associa a una prognosi sfavorevole [22]. Il margine posteriore è più spesso interessato del margine trasversale di resezione e del margine coledocico [23]; un carcinoma distante meno di 1 mm da un margine di resezione deve essere considerato come incompletamente escisso. La presenza o l'assenza di un carcinoma residuo dopo una resezione chirurgica è un fattore prognostico molto importante e sebbene non sia incluso nella stadiazione TNM, esso può essere indicato con il simbolo R (Tab. VII).

Diffusione linfonodale

Il numero totale dei linfonodi regionali asportati dovrebbe essere valutato all'indagine istologica e il numero di linfonodi metastatici e le invasioni perinodali dovrebbero essere riportate. I pazienti con gruppi multipli di linfonodi metastatici sopravvivono significativamente più a lungo dei pazienti con un gruppo singolo di linfonodi metastatici [31]. Il rapporto linfonodale, che è il rapporto tra il numero di linfonodi metastatici e il numero totale di linfonodi esaminati è uno dei più forti fattori predittivi di sopravvivenza dopo chirurgia [28 32].
Per ora l'utilizzo dell'immunoistochimica per individuare micrometastasi nei linfonodi non è raccomandato.

Invasione vascolare

L'invasione dei grossi vasi, comprese l'arteria e la vena mesenterica superiori, la vena porta e/o l'arteria o la vena epatica comune, è un'importante fattore prognostico negativo, dopo resezione [21].

Invasione nervosa

L'invasione perineurale o intraneurale rappresenta un aspetto istologico caratteristico del carcinoma pancreatico. L'invasione neurale intrapancreatica si correla significativamente con l'invasione del plesso extrapancreatico, questa rappresenta una delle cause maggiori di recidiva locale retroperitoneale.

Altri marcatori

Per ora l'uso di tecniche speciali per valutare i marcatori di proliferazione, gli oncogeni (compresi i fattori di crescita e i rispettivi recettori), la poliploidia del DNA o la morfometria nucleare non è giustificata per un utilizzo clinico. Di fatto, si sente il bisogno di marcatori che potrebbero essere usati come indicatori prognostici o utilizzati per trattamenti mirati, come per i cancri mammari, gastrici e polmonari e per alcune neoplasie maligne ematologiche, ma per ora non vi sono test molecolari o test immunoistichimici correlati che rendano possibile un trattamento personalizzato del cancro pancreatico.

Aspetti istologici dell'adenocarcinoma duttale pancreatico [1 4 6]

Il comune ACD è caratterizzato da una proliferazione di ghiandole da piccole a grandi, rivestite da cellule cubiche o alte, disperse in un abbondante stroma desmoplastico. Il grado di formazione di ghiandole è proporzionale al grado di differenziazione e varia da ghiandole ben formate nel carcinoma ben differenziato a cellule singole infiltranti o cellule che formano distese solide in cancri scarsamente differenziati. Quasi tutti gli ADC sono caratterizzati da un'intesa reazione desmoplastica

Tab. VI. Classificazione TNM dei tumori del pancreas AJCC/WHO [25 29].

Tumore primitivo (T)	
TX	Tumore primitivo non definibile
T0	Tumore primitivo non evidenziabile
Tis	Carcinoma in situ compreso PanIN3
T1	Tumore limitato al pancreas di dimensione massima ≤ 2 cm
T2	Tumore limitato al pancreas di dimensione massima > 2 cm
T3	Tumore che si estende oltre il pancreas, ma senza interessamento del tronco celiaco e dell'arteria mesenterica superiore
T4	Tumore che infiltra il tronco celiaco o l'arteria mesenterica superiore
Linfonodi regionali (N)	
NX	Linfonodi regionali non valutabili
N0	Linfonodi regionali esenti da metastasi
N1	Metastasi nei linfonodi regionali
Metastasi a distanza (M)	
MX	Metastasi a distanza non valutabili
M1	Metastasi a distanza assenti
M0	Metastasi a distanza presenti

Raggruppamento in stadi

Stadio	T	N	M
Stadio 0	Tis	N0	M0
Stadio IA	T1	N0	M0
Stadio IB	T2	N0	M0
Stadio IIA	T3	N0	M0
Stadio IIB	T1, T2, T3	N1	M0
Stadio III	T4	Qualsiasi N	M0
Stadio IV	Qualsiasi T	Qualsiasi N	M1

Tab. VII. Tumore residuo dopo resezione chirurgica. Classificazione R.

RX	Presenza di tumore residuo non accertabile
R0	Assenza macroscopica e microscopica di tumore residuo
R1	Presenza di tumore residuo microscopico
R2	Presenza di tumore residuo macroscopico

stromale composta da miofibroblasti, linfociti e cellule infiammatorie. Negli ADC ben differenziati, il pattern di crescita e l'aspetto citologico delle cellule possono essere ingannevolmente benigni, poiché mimano i dottuli non neoplastici delle pancreatiti croniche. Comunque, negli ADC ben differenziati le ghiandole maligne di solito rimpiazzano la normale architettura lobulare degli acini con tubuli organizzati in modo casuale, ma, a basso ingrandimento, l'aspetto lobulare è generalmente conservato nelle pancreatiti croniche, mentre è perso negli ADC ben differenziati. L'immunocolorazione per SMAD4 (DPC4) e TP53 può essere uno strumento utile, quando deve essere fatta la diagnosi differenziale tra le lesioni duttulari rigenerative della pancreatite cronica e l'ADC, perché nelle prime SMAD4 (DPC4) non è mai perso e TP53 è negativo; TP53 è invece spesso positivo negli ADC [4].

Le cellule che rivestono le ghiandole maligne tipicamente formano un singolo strato regolare, ma in alcuni casi possono essere prominenti la stratificazione e le papille irregolari. Il citoplasma delle cellule tumorali può essere abbondante e generalmente contiene una diversa quantità di mucine, in relazione al grado di differenziazione del cancro. I nuclei possono mantenere un orientamento basale negli ADC ben differenziati, ma variano in dimensione, forma e localizzazione intracellulare negli ADC moderatamente e scarsamente differenziati. Le varianti istologiche degli ADC pancreatici sono ben documentate e sono descritte nella classificazione WHO 2010 [29]. Esse sono il carcinoma adenosquamoso, il carcinoma indifferenziato (anaplastico), il carcinoma indifferenziato con cellule simili ad osteoclasti, il carcinoma colloide (mucinoso non cistico), il carcinoma a cellule ad anello con castone, il carcinoma misto duttale-endocrino. La graduazione è fatta in accordo con lo schema di graduazione di WHO (Tab.V) [1 6].

L'ADC pancreatico è una neoplasia particolarmente invasiva che cresce all'interno e lungo i dotti pancreatici, in alcuni casi mima una neoplasia intraepiteliale (PanIN). L'invasione neurale è un reperto molto comune, come anche l'estensione nel tessuto adiposo peripancreatico, dove possono essere viste ghiandole nude [4]. Anche l'invasione dei vasi linfatici ed ematici è un reperto comune nel CP. L'invasione del dotto biliare comune, della parete duodenale e dell'ampolla di Vater è frequentemente osservata nel cancro della testa del pancreas, anche quando la dimensione del tumore è piccola. La formazione di cisti è poco comune nei carcinomi della testa del pancreas, ma può essere osservata nel carcinoma della coda, sollevando un problema di diagnosi differenziale con il tumore cistico mucinoso.

Immunoistochimica nell'adenocarcinoma pancreatico e nelle lesioni precorritrici [4]

L'ACD convenzionale dimostra inevitabilmente almeno una focale positività per le mucine utilizzando l'Alcian Blu da solo o combinato con il PAS. In aggiunta, le colorazioni per le citocheratine (CK) 7, 8, 18 e 19 e per l'antigene epiteliale di membrana (EMA) sono generalmente positive [1 4]. La CK20 è trovata in meno del 10% degli ACD. La CK20 è molto più frequentemente espressa negli adenocarcinomi ampollari (di tipo intestinale, ma non in quelli di tipo pancreatico – biliare), nelle neoplasie intraduttali papillari mucinose (intraductal papillary mucinous neoplasm, IPMN) di tipo intestinale e nel carcinoma colloide (carcinoma mucinoso non cistico) ad esso correlato e nella neoplasia mucinosa cistica con associato carcinoma invasivo. Marcatori non specifici spesso riscontrabili negli ACD comprendono il CA19-9, il CEA, il CA125 e il DUPAN2. Di questi, il CEA e il CA125 sono glicoproteine tumore-associate non espresse dalle cellule duttali normali, ma osservate nelle neoplasie intraepiteliali pancreatiche di basso e alto grado (PanIN). Le proteine MUC sono variamente espresse in tutti i tipi di neoplasie duttali. La maggioranza degli ACD esprimono MUC1 (86%), MUC3, MUC4, MUC5AC (71%). Circa il 20% degli ACD esprimono MUC6 (una mucina delle ghiandole piloriche) e solo il 6% esprimono MUC2. Il CDX2, come il MUC2, è positivo in una minoranza (14%) di ACD usuali, ma è espresso nel 100% dei carcinomi colloidi. Il MUC2 e il CDX2 possono essere utili per differenziare un adenocarcinoma ampollare avanzato da un ACD della testa del pancreas specialmente quando il cancro ampollare è di tipo intestinale: questo tipo si associa ad una positività del 100% per il CDX2. MUC2 e CDX2 non sono mai espressi nella PanIN di basso o alto grado; al contrario una diffusa e intensa positività per MUC2 e CDX2 è osservata nelle IPMN di tipo intestinale, consentendo una distinzione tra questi due tipi di lesioni. Le colorazioni per la cromogranina e la sinaptofisina possono dimostrare la presenza di cellule neuroendocrine sparse associate alle ghiandole neoplastiche. Un'immunocolorazione diffusa per la cromogranina e/o la sinaptofisina suggerisce la possibilità di un carcinoma neuroendocrino (NEC), scarsamente differenziato o di un carcinoma misto adeno – neuroendocrino (mixed adenoneuroendocrine carcinoma, MANEC). Gli ACD iperesprimono fattori di crescita e relativi recettori come il fattore di crescita epidermico (EGF) e i suoi recettori c-erbB-2, c-erbB-3, il fattore trasformante di crescita alfa e beta (TGF alfa e beta) e i relativi recettori, il fattore di crescita derivato dalle piastrine (PDGF) A e B e i loro recettori e il fattore fibroblastico di crescita e il suo recettore [4].

Lesioni premaligne [2 4 29]

Diverse lesioni premaligne, non invasive, possono dare origine ad un adenocarcinoma invasivo del pancreas [2 4 29]. La scoperta precoce di queste lesioni non invasive offre la possibilità di una cura di carcinomi pancreatici iniziali e di ridurre la mortalità per cancro. Le lesioni premaligne dell'ACD invasivo comprendono una lesione microscopica: la neoplasia pancreatica intraepiteliale (PanIN), e due lesioni che formano massa: la neoplasia intraduttale papillare mucinosa (IPMN) e la neoplasia mucinosa cistica (MCN). La loro scoperta clinica e il trattamento possono interrompere la progressione a carcinoma invasivo [33].

Neoplasia pancreatica intraepiteliale (PanIN) [2 4 29]

La PanIN è definita come una neoplasia epiteliale microscopica, papillare o piatta, non invasiva, che origina nei dotti pancreatici. Le PanIN sono caratterizzate da cellule da colonnari a cubiche con varie quantità di mucine e gradi di atipia citologica e architetturale. Le PanIN di solito interessano dotti di diametro minore di 5 mm [2 4 29]. Le lesioni PanIN (comprese le lesioni prima denominate iperplasie duttali non papillari o papillari) si trovano caratteristicamente nei dotti intralobulari, non sono identificabili macroscopicamente e sono clinicamente silenti. Le PanIN sono suddivise in tre gradi in base al grado di atipia citologica e architetturale. Le lesioni con atipia minima, moderata o marcata sono indicate come PanIN-1, PanIN-2 e PanIN-3, rispettivamente. Le lesioni PanIN-1 sono suddivise in piatte (PanIN-1A) e papillari (PanIN-1B). Le lesioni PanIN sono state integrate in un modello di progressione dell'ADC che lega le variazioni morfologiche dell'epitelio duttale alle alterazioni genetiche. Il profilo genetico delle PanIN mostra sia l'attivazione di oncogeni che l'inattivazione di geni oncosoppressori.
Le mutazioni puntiformi attivanti del gene KRAS si verificano nelle lesioni premaligne di grado più basso (PanIN-1), collocandole tra gli eventi genetici più precoci che si verificano nello sviluppo dell'ACD. La perdita dell'espressione della proteina p16 è osservata in meno del 30% delle PanIN di basso grado (PanIN-1), nel 55% delle PanIN-2 e nel 70% delle PanIN-3. L'inattivazione del gene TP53 appare come evento relativamente tardivo nello sviluppo del cancro pancreatico, visto che compare prevalentemente in lesioni premaligne di alto grado (PanIN-3).
L'informazione relativa alla progressione dei diversi tipi di PanIN è per ora limitata. Il potenziale maligno delle PanIN-1 e PanIN-2 non è attualmente dimostrato e non è richiesto ai patologi di riportare queste lesioni nei referti. Sebbene il significato clinico delle PanIN-3 non sia chiaramente stabilito, essere dovrebbero essere riconosciute e riportate nella diagnosi anatomo-patologica.

Neoplasia intraduttale papillare mucinosa (IPMN) [4 29]

La IPMN è caratterizzata da una proliferazione intraduttale di cellule colonnari mucipare, macroscopicamente visibile (tipicamente maggiore di 1 cm) e originata nel dotto pancreatico principale o in una delle principali ramificazioni. I gradi di formazione di papille, secrezione di mucine, dilatazione dei dotti (formazione di cisti) e di displasia sono variabili [20 29]. Le IPMN non hanno lo stroma periduttale, ipercellulare, di tipo ovarico, che caratterizza le neoplasie mucinose cistiche. Le IPMN non invasive sono classificate sulla base del grado di atipia cito-architetturale in tre gradi: con displasia di basso grado, di grado intermedio o di alto grado – carcinoma in situ [4 29]. Quando le IPMN sono associate a un carcinoma invasivo, esse devono essere classificate a parte.
Esse formano un gruppo eterogeneo di neoplasie e possono essere suddivise in almeno quattro tipi sulla base della loro morfologia e dell'immunofenotipo delle mucine.

1. *Tipo gastrico*: si trova caratteristicamente nelle diramazioni duttali. L'epitelio che riveste le IPMN di tipo gastrico è generalmente di tipo foveolare, mostra un grado di atipia basso o moderato e esprime, immunoistochimicamente MUC5AC, ma non MUC1 o MUC2 (possono essere presenti solo sparse cellule caliciformi)
2. *Tipo intestinale*: caratterizzato dall'interessamento del dotto principale e dalla formazione di papille alte, rivestite da cellule colonnari, con nuclei allungati, pseudostratificati e con citoplasma basofilo e vario contenuto di mucine apicali, simili agli adenomi villosi del colon. Esse hanno generalmente un grado di displasia moderato o alto e sono immunoreattive per MUC2 e CDX2.
3. *Tipo pancreatico-biliare*: è meno frequente degli altri tipi, interessa caratteristicamente il dotto pancreatico maggiore ed è caratterizzato da papille ramificate con una displasia ad alto grado. Le cellule dell'IPMN di tipo pancreatico-biliare esprimono MUC1, ma non MUC2 o CDX2.
4. *Tipo oncocitario*: è caratterizzato dall'interessamento del dotto pancreatico principale o delle sue ramificazioni maggiori ed è formato da papille rivestite da 2 – 5 strati di cellule cuboidi con abbondante citoplasma eosinofilo e granuloso. Il MUC6, una mucina di tipo pilorico, e l'HepPar1 (Hepatocyte-Paraffin-1) sono regolarmente e diffusamente espressi, mentre MUC1, MUC2, MUC5AC e CDX2 sono negativi o solo focalmente presenti.

Mentre la comune IPMN di tipo intestinale, MUC2+, può essere considerata come il precursore del carcinoma colloide (carcinoma mucinoso non cistico) MUC2+, la IPMN di tipo pancreatico-biliare, MUC2-/MUC1+, sembra avere una stretta relazione con il tipo comune di ACD (il profilo di espressione immunoistochimica delle mucine distingue i diversi tipi di IPMN e stabilisce i loro rapporti con il carcinoma colloide e l'ACD [34]). Le IPMN associate a un carcinoma invasivo di tipo colloide hanno una prognosi migliore rispetto a quelle associate ad un cancro invasivo di tipo duttale (tubulare).
Mutazioni puntiformi attivanti dell'oncogene KRAS sono state riportate nel 30 – 80% delle IPMN, con prevalenza nelle IPMN ad alto grado. Mutazioni del gene PIK3CA, che sono presenti anche nei carcinomi colloidi, si riscontrano in circa il 10% delle IPMN, ma sono assenti negli ACD ordinari.

Neoplasia mucinosa cistica (MCN) [19 29]

È una neoplasia che colpisce quasi esclusivamente le femmine, interessa prevalentemente la coda del pancreas, non comunica con il sistema duttale ed è generalmente accompagnata da un caratteristico stroma di tipo ovarico [19 35]. L'infiltrazione carcinomatosa dello stroma caratterizza la MCN con carcinoma invasivo associato. La componente invasiva assomiglia solitamente all'ADC ordinario.
Come nello sviluppo dell'ADC, le mutazioni di KRAS rappresentano eventi precoci, mentre l'inattivazione di

p53 e di DPC4 è un'alterazione genetica relativamente tardiva nella progressione di una MCN non invasiva a una MCN invasiva [36 37].

Suscettibilità genetica e patologia molecolare

Carcinoma pancreatico familiare [1 5]

Il cancro pancreatico è una malattia causata da mutazioni ereditarie (germinali) o acquisite (somatiche) in geni coinvolti nella patogenesi del cancro. Le basi genetiche della maggior parte dei casi familiari (80%) sono conosciute. L'aumento di rischio del CP è ben documentato in alcune sindromi genetiche ereditarie quali la sindrome del carcinoma mammario ereditario e di altri geni dell'anemia di Fanconi (mutazioni di BRCA2, PALB2, FANC-C, FANC-G e probabilmente BRCA1); la sindrome del nevo displastico e melanoma (FAMMM), da mutazioni di p16; la sindrome di Peutz-Jeghers (mutazioni di STK11/LKB1); la pancreatite cronica ereditaria (mutazioni di PRSS1); la sindrome di Lynch o del carcinoma colorettale ereditario non associato a poliposi (HNPCC), con mutazioni germinali dei geni di riparazione dell'accoppiamento del DNA e la sindrome del carcinoma pancreatico familiare (tre o più familiari con un carcinoma pancreatico), il cui gene è ancora sconosciuto [1 4 5] (Tab. VIII). Per i pazienti che hanno queste alterazioni molecolari o caratteristiche genetiche, si deve effettuare un'attenta sorveglianza nel tempo utilizzando l'EUS e la RM per una diagnosi precoce del CP [38]. L'informazione sulle alterazioni genetiche ereditarie può essere utilizzata anche per quantificare il rischio del soggetto, portatore di mutazione, di sviluppare cancri extra-pancreatici e per fare uno screening di queste neoplasie. Al contrario, un programma di screening non è indicato per individui che hanno un rischio nella media (*United States Preventive Service Task Force*).

Alterazioni genetiche somatiche nell'adenocarcinoma pancreatico sporadico [1 4]

Più del 90% degli ACD sono neoplasie sporadiche con un'attivazione dell'oncogene KRAS, da mutazioni puntiformi nel codone 12. Le mutazioni di TP53 si trovano nel 60% degli ACD sporadici. La maggior parte degli ACD hanno anche anomalie di p16/CDKN2A, dovute sia a mutazione (75-85%) o a silenziamento da ipermetilazione del promotore (15%). La perdita di SMAD4 (DPC4) è stata trovata nel 60% degli ACD invasivi [1].
Recentemente si è trovato che l'inattivazione di SMAD4 (DPC4), sia per mutazione intragenica che per delezione omozigote, è un marcatore prognostico sfavorevole indipendente, anche quando è valutato tenendo conto dello stato linfonodale, del grado, dello stato dei margini, delle dimensioni del tumore e dell'età del paziente [39]. Al contrario, l'inattivazione del gene p16/CDKN2A, la mutazione di TP53 e l'attivazione di KRAS non correlano con la sopravvivenza, in un'analisi multivariata. Iacobuzio-Danahue et al. [40] hanno dimostrato che pazienti con un cancro con una perdita di SMAD4 (DPC4) in genere muoiono con una malattia metastatica diffusa, mentre i pazienti con ACD con gene SMAD4 (DPC4) normale muoiono più frequentemente con una malattia localizzata. Quindi la marcatura immunoistochimica di SMAD4 (DPC4) può essere utilizzata per separare due sottotipi molecolari di CP e per accertare l'origine pancreaticobiliare di un carcinoma metastatico, poiché solo rari carcinomi gastrointestinali mostrano una perdita della proteina SMAD4 (DPC4) [1].
Jones et al. [41] riportano del sequenziamento di 23219 trascritti in una serie di CP; le alterazioni genetiche apparivano concentrarsi su un gruppo centrale di 12 vie cellulari di segnale che erano alterate nel 65-100% delle neoplasie. Le vie di segnale coinvolte comprendevano: l'apoptosi, il controllo del danno del DNA, la regolazione della trascrizione della fase G1/S, la via di segnale hedgehog, l'adesione cellulare omofilica, i sistemi di segnalazione dell'integrina, del KRAS, del TGFβ e di wnt/notch e la regolazione dell'invasione. Questi dati potrebbero essere utili, in futuro, per una classificazione molecolare del cancro pancreatico e per una terapia orientata su bersagli molecolari.

Utilizzo clinico delle alterazioni genetiche del carcinoma pancreatico [1]

Le alterazioni genetiche ereditarie possono essere utilizzate per valutare il rischio di sviluppare un CP in una persona e i soggetti ad alto rischio possono beneficiare di un follow-up stretto per individuare i CP iniziali e addirittura lesioni premaligne non invasive. Tutti gli

Tab. VIII. Sindromi genetiche associate a un aumentato rischio di carcinoma pancreatico

Sindrome	Geni (localizzazione cromosomica)	Rischio di neoplasie in altre sedi	Rischio di CP all'età di 70 anni
Sindrome del carcinoma mammario ereditario e di altri geni dell'anemia di Fanconi	BRCA2 (13q), PALB2 (16p), FANC-C (9q), FANC-G (9p) e probabilmente BRCA1 (17q)	Mammella, ovaio, prostata	3,5 – 10% per BRCA2
Sindrome del nevo displastico e melanoma (FAMMM)	P16/CDKN2A (9p21)	Melanoma	15%
Sindrome di Lynch o del carcinoma colorettale non poliposico (HNPCC)	MSH2 (2p), MLH1 (3p) e altre	Gastrointestinale, endometrio, ovaio	< 5%
Pancreatite cronica ereditaria	PRSS1 (7q35), SPINK1 (5q)	Nessuno	40%
Sindrome di Peutz-Jeghers	STK11/LKB1 (19p)	Gastrointestinale, mammella	30 – 60%
Carcinoma pancreatico familiare (3 o più pazienti con CP)	Sconosciuto		9 – 38% (80 anni)

ACD sono morfologicamente simili anche quando hanno profili molecolari diversi, di conseguenza solo la comprensione del profilo genetico dei CP potrà avere applicazioni cliniche dirette per valutare la possibilità di terapie mirate, in un prossimo futuro. Ad esempio, la mitomicina e gli inbitori della poli (ADP-riboso) polimerasi (PARP) potebbero essere particolarmente efficaci nel trattare CP con mutazione del gene BRCA2 (che sono morfologicamente identici agli ADC ordinari, senza mutazione di BRCA2) e è stato proposto che la L-alanosina e altri inibitori della via di salvataggio della sintesi di AMP possano essere particolarmente efficaci nel trattamento dei CP con delezione omozigote di p16/CDKN2A. I carcinomi midollari del pancreas mostrano spesso una instabilità dei microsatelliti (MSI) e, sulla base delle osservazioni sui carcinomi colorettali e su dati preliminari su CP, sembra verisimile che la terapia a base di 5-fluorouracile (5-FU) non sia di beneficio per i pazienti con CP con MSI [1].

Lista di controllo (CHECKLIST)

Controllo dell'informazione clinica e della completezza dei dati clinici

Esame macroscopico

Campione inviato
- Duodenopancreasectomia parziale o totale
- Resezione distale
- Altro

Tumore
- Sede con riferimento al dotto pancreatico principale e alla papilla di Vater
- Dimensioni (in cm)
- Aspetto del tumore
  - Solido (diffuso, nodulare, lobulato, emorragico, necrotico)
  - Cistico (uniloculare, multiloculare, con lesioni intraduttali); contenuto cistico (mucoide denso o fluido, sieroso, ematico); comunicazione delle cisti con le ramificazioni duttali
  - Componente stromale (sclerotica, non sclerotica)
  - Margini tumorali: espansivi – infiltrativi
  - Colore e consistenza del tumore (marrone chiaro, bianco, bruno, rosso, giallo, variegato; molle, carnoso, duro, scirroso, friabile, spugnoso)
- Invasione dei tessuti / organi vicini
- Invasione dei grandi vasi

Lesioni dei tessuti non cancerosi
- Lesioni duttali (ostruzioni, calcificazioni, cisti)
- Lesioni parenchimali (fibrosi, etc.)
- Lesioni della parete duodenale

Linfonodi peripancreatici

Segmenti vascolari (allegati)

Margini di resezione

Valutazione e documentazione della distanza e dei rapporti tra il tumore e i seguenti margini:
- Margine coledocico
- Margine parenchimale pancreatico con il dotto principale
- Margini parietali duodenali (prossimale e distale)
- Margini di resezione circonferenziali
  - Anteriore
  - Posteriore
  - Mediale (doccia mesenterica)

Linfonodi regionali

I linfonodi regionali suddivisi secondo le norme TNM in:
- Superiori
- Inferiori
- Anteriori
- Posteriori
- Splenici
- Celiaci

Esame microscopico

Tumore
- Tipo istologico
- Grado istologico
- Estensione dell'invasione
  - Nei tessuti / organi adiacenti (vedi anche esame macroscopico)
  - Nei vasi sanguigni
  - Nei vasi linfatici
  - Invasione perineurale

Interessamento linfonodale
- Numero per gruppo (totale / positivi) e estensione nei tessuti perilinfonodali

Margini di resezione
- Estensione / tipo di invasione (invasione dei vasi linfatici e /o ematici; disseminazione delle cellule tumorali)

Pancreas peritumorale: pancreatite, metaplasia, neoplasia intraepiteliale

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In memoriam of John G. Azzopardi

The international community of pathologists has recently lost one of its icons, John G. Azzopardi. He was born in Valetta (Malta) on June 25th 1929 and died on January 2nd 2013 in London (UK). He has been laid to rest in Sliema (Malta).

He started his medical training at the Royal University of Malta in 1942 at the tender age of 13. Lectures were scattered in time and space because of the siege of Malta during the Second World War. Hospital training was under wartime emergency conditions. He qualified as M.D. in 1949, standing first in his class. He then moved to England where he spent the first years in junior house jobs in Sheffield, and after took a scholarship to attend a course on Pathology at the Royal Postgraduate Medical School (RPMS), Hammersmith Hospital, London. Apart from brief sabbaticals, he never left "his" hospital. He was appointed to the academic hospital staff,and rose through the ranks from junior posts to Lecturer, Reader and Professor of Oncology until retirement.

He was invited to spend a year (1960-61) at the prestigious Armed Forces Institute of Pathology, Washington, DC, as well as two months at the University of Bologna in 1972. He gave several well-received lectures in various European countries, but he was fully "discovered" by the North American Pathologists in 1975, when he was invited to speak and give a slide seminar at the annual California Tumor Registry at Stanford University. This visit resulted in several job offers in pursuing an academic career in the United States. While he was thrilled by these offers, he never left the RPMS as he did not like changes nor did he look for honours. Interestingly, he has never compiled his CV; the reason given by those who have worked with him was that 'he was the sort of man that did not need one".

Mentioning Azzopardi's name results in an immediate association with breast pathology. However, John G. Azzopardi was far from being a pure specialist and he can be included in the general surgical pathologist-morphologist-pathobiologist species, which has flourished in Europe, to use J. Rosai's words [1]. The majority of the papers he has written have become the standard reference for the respective entities: the schwannian origin of myoblastoma [2]; the mucin profile of salivary gland tumours [3]; the insuperable description of bronchial oat-cell carcinoma with DNA incrustration of the wall of blood vessels (since then known as the Azzopardi's phenomenon) [4]; the genesis of adenolymphoma of parotid in lymph nodes [5]; the neuroendocrine (divergent) differentiation in gastric [6], cervical [7], prostatic [8] and breast carcinomas [9 10]; the retrogression in testicular seminomas with viable metastases [11]; the systemic effects of neoplasia (paraneoplastic syndromes) [12]; the pathology of "non-endocrine tumours" associated with Cushing syndrome [13]; the distinctive tumour entity of bone and soft tissue associated with acquired vitamin-D-resistant osteomalacia [14]; and the occurrence of blue nevi in the capsule of lymph nodes [15], to cite a few. As impressive as this work is, it pales in comparison with his magnum opus, the book "Problems in Breast Pathology", published in 1979 (Volume 11 in the series Major Problems in Pathology, Bennington JL) [1].

Even today, it is still regarded as the best and most insightful work on the morphologic analysis of breast tumours, and as a book which laid the foundation for subsequent publications. The masterful histologic descriptions are combined with clear definition of entities. The critical analysis of the literature is presented in an admirable "reader-friendly" fashion. In the preface of the book Azzopardi states: "all the references, unless otherwise stated, have been read in their entirety, many of them more times than I care to remember". James Bennington (consulting editor of the series) predicted this book would become "an indispensable and timeless reference for all those who are interested in the surgical pathology of breast tumours". Thirty years later, an issue of Seminars in Diagnostic Pathology entitled "Problems in breast pathology revisited" was written by some of those who had worked directly with him or had been influenced by his unique insights to the field.

Most of his trainees learned that in order to work with Professor Azzopardi the following simple "rules" had to be respected: 1. adhere strictly to the official starting time; 2. complete the requested task with accuracy and celerity; 3. during the consult sessions, not to speak until asked to address the question "What's the story?" Start with the age followed by the gender of the patient; 4. not to carry histologic slides (even if it is only one) in hand or pockets, but to place them on slide trays, with the lid closed. Once these rules were followed, one would then discover a fatherly teacher, a generous friend, and sometimes target of one of his abrasive but well intentional remarks. To a famous professor of pathology, expert in morphometry, who asked him what he thought of that technique, he replied that he liked it very much, provided he was not involved with it. On another instance, a young pathologist showed him a tumour case accompanied by the introductory remark "I do not know what this is but would diagnose it as benign". Professor Azzopardi looked at the poor pathologist with a sight of unforgettable commiseration and let him know that it was very

dangerous to label a tumour as benign or malignant if one did not know its nature.
Professor Azzopardi has been consulted by pathologists from all over the world, and provided expert opinion free of charge. He kept the most educational and diagnostically challenging cases in "black slide boxes", accompanied by his handwritten notes containing underlined key points organized perfectly for future studies. Most of this highly instructive histologic slide collection is currently available in the Department of Pathology at the University of Bologna.
A meeting of breast pathology in Professor Azzopardi's honour was held in Malta in May 2006. A large audience/speakers from all parts of the world convened, including several of his pupils that he used to call "his stable" as well as pathologists who wanted to meet Professor Azzopardi for the first time. To quote a South American pathologist, "meeting him was an unforgettable experience".
Professor J.G. Azzopardi is survived by his wife Sally, who lovingly typed the entire book, in the pre-computer era, as he did not trust anybody else with such a task) two children (Timothy and Joanna), and four granddaughters.

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Errata

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Case report

Biphasic large cell neuroendocrine carcinoma – pure mucinous carcinoma of the gallbladder (MANEC): a unique combination

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Corrige

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