June 19, 2002

7:45 am	Registration
9:00 am	- Welcoming Address: *S.E. Rev.ma Mons. R. Ruotolo, President.* - A Brief Summary of Padre Pio's Life and His Hospital: *O. Pennelli, Medical Director.* - Introduction to the Symposium: *Michele Bisceglia, Chief Pathologist.* IRCCS "Casa Sollievo della Sofferenza" Hospital
9:30 am	The Arkadi M. Rywlin International Slide Seminar - Homage to a Master of Pathology *S. Suster*
10:00 am	Diffuse Large B-cell Lymphoma: Morphologic, Phenotypic, Molecular and Clinical Features *S. Pileri*
10:45 am	COFFEE BREAK
11:15 am	The Role of Electron Microscopy in the Diagnosis of Soft Tissue Tumors *B. Eyden*
12:00 am	Surgical Pathology -Session I: Soft Tissue & Bone Case Presentations (4 cases) *AMR Seminar Club Members*
1:20 pm	LUNCH
3:00 pm	Surgical Pathology –Session II: Urogenital tract I. Case Presentations (4 cases) *AMR Seminar Club Members*
4:20 pm	COFFEE BREAK
4:50 pm	Surgical Pathology –Session III: Urogenital tract II. Case Presentations (5 cases) *AMR Seminar Club Members*
6:30-7:30 pm.	Quiz Cases (Illustrated Exhibition)

DIFFUSE LARGE B-CELL LYMPHOMA: ONE OR MORE ENTITIES? PRESENT CONTROVERSIES AND POSSIBLE TOOLS FOR ITS SUBCLASSIFICATION.

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Diffuse large B-cell lymphoma (DLBCL) is a neoplastic entity, which was introduced by the REAL Classification (Harris 1994) and has been maintained in the recently developed WHO scheme (Jaffe 2001). It accounts for about 30% of all lymphomas in Western Countries (Anon. 1997) and represents an heterogeneous group of tumours on morphologic, phenotypic, molecular, and clinic grounds. The process may develop *de novo* or derive from a previous indolent lymphoma, such as B-cell chronic lymphocytic leukemia, immunocytoma, marginal zone lymphoma or follicular lymphoma (FL). In the last few years, DLBCL has been the object of an increasing number of clinic, pathologic and molecular studies, whose results will be briefly discussed in the following.

MORPHOLOGY

On morphologic grounds, DLBCL consists of large cells (mean diameter ≥ 20 μm), more often characterised by pronounced nuclear pleomorphism, prominent nucleoli, and a rim of basophilic cytoplasm. At low power view, the neoplasm grows diffusely, sometimes spreading through residual sinuses at the lymph node level. Mitotic figures are always numerous. Macrophages phagocytizing nuclear debris can be encountered. In a percentage of cases, DLBCL evokes a fibrotic reaction, which may become prominent in the retroperitoneum (Pileri 2001) and mediastinum (Pileri 2002a): in the latter anatomic site, the process displays peculiar clinico-pathologic features which justify its identification as a variant of DLBCL (see below). Occasionally, the tumour may be confined to the lumina of the small vessels: this finding corresponds to another clinico-pathologic variant of DLBCL, termed "intravascular large B-cell lymphoma" (Jaffe 2001) (see below). At high power view, in 20-25% of cases one cytotype predominates over the others, thus allowing the subclassification of the process into: centroblastic, immunoblastic, anaplastic, plasmablastic, and with large multilobated nuclei

(Tab. 1). Interestingly enough, the plasmablastic variant is frequently observed in the oral cavity of HIV-positive patients (Jaffe 2001, Flaitz 2002). A further cytological variant of DLBCL, termed " histiocyte-rich/T-cell-rich B-cell lymphoma", is characterised by a high content of reactive T-lymphocytes and/or histiocytes: it can be confused with lymphocyte predominant or classical lymphocyte-rich Hodgkin's lymphoma (Table 2) (Ramsay 1988, Chittal 1991, Delabie 1992, Harris 1994, Jaffe 2001, Achten 2002, Torlakovic 2001, Jundt 2002, Pileri 2002b). This variant occasionally shows prominent angiocentricity, thus producing the picture originally described as "lymphomatoid granulomatosis" (Guinee 1994, Jaffe 2001). Finally, in cases with prominent sclerosis lymphomatous cells may become spindle-shaped, thus mimicking a mesenchymal growth (Chim 2001, Nozawa 2001). There is no consensus on the usefulness of distinguishing cytological subtypes of DLBCL (Engelhard 1997, Baars 1999). A recent report of Diebold et al. (Diebold 2002) based on the application of the updated Kiel Classification (Lennert & Feller 1992) to 444 DLBCLs has shown that "no significant differences in survival were found between the three major groups (centroblastic, immunoblastic and anaplastic)". The same authors found that "the 5-year overall survival and failure free survival of patients with DLBCL, not containing immunoblasts was … better than the survival of those containing immunoblasts" (Diebold 2002). Nevertheless, this morphologic approach – which is based on subjective and not always reproducible criteria (Anon. 1997) – needs the integration of phenotypic and molecular data, if one aims to provide the clinician with information actually useful for prognosis and therapy.

PHENOTYPE

On phenotypic grounds, neoplastic cells do express a series of B-cell associated antigens, such as CD19, CD20, CD22, CD79a, and BSAP (B-cell Specific Activating Protein) (Pileri 2000). Interestingly, the leukocyte common antigen/CD45 is absent in about 30% of DLBCLs of the immunoblastic and anaplastic types (Falini 1990, Harris 1994, Pileri 2000, Jaffe 2001). The *BCL-10* gene product has been detected in about 50% of the extranodal tumours (Ohshima 2001). Based on the expression of CD10 and Bcl-6 protein, three main groups of DLBCL have been distinguished: $CD10^+/Bcl\text{-}6^+$, $CD10^-/Bcl\text{-}6^+$, and $CD10^-/Bcl\text{-}6^-$ (Carbone 1998, Dogan 2000, Pileri 2000, Ree 2001). The first group corresponds to neoplasms which derive from germinal centre cells (GCCs) - either *de novo* or following transformation of a pre-existing FL - and are usually characterised by Bcl-2 protein expression. The remaining two categories appear more heterogeneous. In fact, they include tumours with the following profiles: a) $Bcl\text{-}6^+/CD10^-/Bcl\text{-}2^+$ [possibly also derived from GCCs, by considering that CD10 expression may be weak to negative in grade 3 FL (Eshoa 2001)], b) $Bcl\text{-}6^+/CD10^-/Bcl\text{-}2^-$ (with controversial histogenesis), c) $Bcl\text{-}6^-/CD10^-/Bcl\text{-}2^{-/+}/CD20^{-/+}/CD79a^+/CD30^{-/+}/EMA^{+/-}/CD138^+/VS38c^+/CIg^+$ (with immunoblastic morphology), and d) $Bcl\text{-}6^-/CD10^-/Bcl\text{-}2^-/CD20^+/CD79a^+/CD30^+/EMA^{+/-}/CD138^-/VS38c^-$ (with anaplastic morphology). Great interest has recently been aroused by the *MUM-1* gene product, also known as Interferon Regulating Factor 4 (IRF4): under physiologic conditions, this molecule is expressed by B-cells, that have already overcome GC selection, have lost Bcl-6 protein expression and are on the way to leave the GC and to mature to plasma cells (Falini 2000, Pileri 2000, Falini & Mason 2002). It has been proposed that the combination of Bcl-6, IRF4 and CD138 might assist in distinguishing among normal and neoplastic GCCs ($Bcl\text{-}6^+/IRF4^-/CD138^-$), late GC/early post-GCCs ($Bcl\text{-}6^-/IRF4^+/CD138^-$), and post-GCCs ($Bcl\text{-}6^-$, $IRF4^+/CD138^+$) (Carbone 2001). Although these concepts have successfully been applied to the subclassification of malignant lymphomas in HIV^+ patients (Carbone 2001), it is unclear whether they pertain DLBCLs of the immunocompetent host. In fact, the latter often display co-expression of the IRF4 and Bcl-6 molecules (Falini 2000, Pileri 2000, Falini & Mason 2002, Pileri 2002). A study carried out on tissue

micro-arrays has revealed that IRF4 staining lacks specificity in detecting plasmacytic differentiation as compared to CD138 (Natkuman 2001). Another phenotypic feature which has recently merited attention is CD5 expression in DLBCLs. This finding has been observed by Yamaguchi et al. (Yamaguchi 2002) in 109 *de novo* cases, supposed to represent a unique subgroup of DLBCLs because of the uniform phenotype ($CD5^+/CD10^-/CD19^+/CD20^+/CD21^+/CD23^-$ /cyclin D1-), usual centroblastic morphology and aggressive clinical behaviour. In this setting, the lack of cyclin D1 is diagnostically relevant: in fact, it allows the distinction between $CD5^+$ DLBCLs and polymorphic mantle cell lymphomas, which also consist of large cells, but regularly carry the t(11;14) translocation and *BCL-1* gene over-expression (Pileri 2000, Jaffe 2001). Besides CD5 positivity, several other markers have been proposed as prognostically relevant: among these Bcl-2, CD10, Bcl-6, cyclin D3, cyclin B, and survivin. In most studies, Bcl-2 protein expression – alone or in combination with other factors (e.g. CD10 positivity or high IPI) – is regarded as an evidence of t(14;18) and an aggressive clinical course, the overall survival and disease-free survival curves being significantly worse than in Bcl-2 negative cases (Hill 1996, Gascoyne 1997, Kramer 1998, Ohshima 2001, Xu 2001, Barrans 2002 a,b). The relevance of CD10 staining is controversial: in fact, while Xu et al. (Xu 2001) reported $CD10^+$ phenotype associated with a significantly lower rate of complete remissions, Ohshima et al. (Oshima 2001), Barrans et al. (Barrans 2002 a,b) and Huang et al. (Huang 2002) considered CD10 expression (possibly in association with Bcl-6 protein) as a favourable prognostic factor, in keeping with the postulated GCC derivation of the tumour and results of DNA micro-array studies (see below). Expression of cyclin D3 in more than 50% of the neoplastic cells seems to correlate with more advanced stage and widespread extranodal disease at presentation, as well as with lower complete response rate and shorter overall survival (Filipits 2002). Analogously, the co-expression of cyclin B and survivin might herald a more aggressive clinical course by facilitating the G_2/M transition of the neoplastic cells (Kuttler 2002).

GENOTYPE

Numerous cytogenetic and molecular aberrations have been detected in DLBCLs: among these, some display a higher incidence, such as the abnormalities involving the *BCL-2*, *BCL-6*, and *c-MYC* genes, +5, +6, +7, +18, $6q^{del}$, and the breakpoints at 1q2-23, 6q21-25, and 14q11-12 (Lo Coco 1994; Hill 1996; Gascoyne 1997; Kramer 1998; Vitolo 1998; Jerkeman 1999, Capello 2000, Pileri 2000, Jaffe 2001, Huang 2002). These aberrations – not always associated with over-expression of the corresponding gene product (Hill 1996, Gascoyne 1997, Kramer 1998, Falini & Mason 2002) – have been the object of numerous studies aiming to assess their potential prognostic value, which have often produced conflicting results. About a decade ago, Ye et al. (Ye 1993), Offit et al. (Offit 1994) and Lo Coco et al. (1994) claimed that DLBCLs carrying *BCL-6* gene rearrangement due to a translocation involving the 3q27 locus had a more favourable course than those lacking this alteration and/or showing t(14;18). The latter finding was regarded as indicative of the fact that DLBCLs of GCC derivation had a poor response to therapy. More recently, however, it has become evident that *BCL-6* gene abnormalities represent a much more complex issue. Akasaka et al. (Akasaka 2000) reported that a translocation involving the IgV_H and *BCL-6* gene loci was associated with a good prognosis, while the formation of a fusion gene between *BCL-6* and a different non-Ig partner was not. By real time RT-PCR, Lossos et al. (Lossos 2001) displayed that high *BCL-6* gene expression correlated with a favourable outcome, while low expression levels were detected in patients with relapsing or resistant disease. Barrans et al. (Barrans 2002a) suggested that the association among *BCL-6* gene rearrangements, Bcl-2 protein expression and a non-GC phenotype might represent an unfavourable prognostic indicator. Finally, Vitolo

et al. (Vitolo 2002) and Artiga et al. (Artiga 2002) have respectively shown that point mutations at the 5' non-coding regions of the *BCL-6* proto-oncogene and short mutational hot spots in the first intron of *BCL-6* gene do occur in patients with prolonged 5-year disease-free and failure-free survival. Concerning new prognostic markers, Esteller et al. (Esteller 2002) have recently evidenced that promoter hypermethylation of the O(6)-methylguanine DNA methyltransferase (MGMT) in DLBCL is associated with statistically significant increase in overall survival and progression-free survival. In the Nature issue of February 3 2000, Alizadeh et al. (2000) first reported on the application to DLBCL of the c-DNA micro-array techniques, which assess the expression level of thousand genes by employing the m-RNA extracted from frozen samples. In particular, they proposed that the diversity in gene expression among DLBCLs apparently reflected the variation in tumour proliferation rate, host response and differentiation state of the tumour, and that patients with a GC B-cell-like signature might run a more favourable course than those with an activated B-cell-like profile (Alizadeh 2000). The same Group further strengthened their proposal by showing that GC B-cell-like DLBCLs (all associated with a good response to therapy) carried IgV_H gene ongoing mutations, CD10 expression and – possibly – t(14;18), as expected in tumors derived from GCCs (Lossos 2000, Huang 2002). These findings are indeed opposite to concept widely diffused in the literature (Ye 1993, Offit 1994, Lo Coco 1994, Hill 1996, Gascoyne 1997, Kramer 1998, Ohshima 2001, Xu 2001, Barrans 2002a,b) that the occurrence of t(14;18) and possible GCC derivation could identify a subgroup of uncurable DLBCLs. In February 2002, by adopting the oligonucleotide micro-array strategy and supervised learning prediction method, Shipp et al. (Shipp 2002) have shown that the outcome of patients with DLBCL, treated with CHOP-based chemotherapy, might be influenced by gene expression profiles other than the histogenetic ones. Genes of prognostic value seem to include some that regulate responses to B-receptor signaling, critical serine/threonine phosphorylation pathways and apoptosis (Shipp 2002).

CLINICAL FEATURES

DLBCL can be observed at any age, the median age being 64 years, without sex prevalence (Armitage 1998). It presents more often at the nodal level, although extranodal primary locations are not infrequent (Armitage 1998). The process can develop *de novo* or arise within the context of a pre-existing indolent form (Harris 1994, Pileri 2000, Jaffe 2001). According to the presentation modalities, some clinical varieties of the process can be recognised (Table 3): these will be treated in the following. The prognosis depends on the combination of clinical parameters (such as the stage, international prognostic index or performance status), phenotype and molecular characteristics (see above). During staging procedures, a discordant histotype is found in about 30% of DLBCLs, because of the occurrence of an indolent component at the bone-marrow level: this finding does not modify the prognosis, although a higher prevalence of FL recurrences is expected (Fisher 1981, Conlan 1990, Robertson 1991, Cabanillas 1992, Hodges 1994).

PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA

Primary mediastinal (thymic) B-cell lymphoma (PMBL) is a distinct clinico-pathologic entity, originally described in 1980 (Lichtestein 1980), officially acknowledged in the REAL Classification in 1994 (Harris 1994) and more recently included in the WHO book on the tumors of the hematopoietic and lymphoid tissues (Jaffe 2001). In the literature, it has been the object of numerous reports, which have provided conflicting results in terms of phenotype and molecular characteristics, thus preventing a definitive histogenetic interpretation (Addis & Isaacson 1986, Menestrina 1986, Isaacson 1987, Scarpa 1987, Möller 1987, Aisenberg 1988, Lamarre 1989, Al-Sharabati 1991, Scarpa 1991,

Eichelmann 1992, Falini 1995, Kanavaros 1995, Joos 1996, Tsang 1996, Higgins and Warnke 1999, Copie-Bergman 1999, Capello 2000, Van Besien 2001, Riguad 2001, de Laval 2001, Laithäuser 2001, Palanisamy 2002)

On clinical grounds (Perrone 1986, Menestrina 1986, Lazzarino 1993, Falini 1995, Cazals-Hatem 1996, Abou-Elella 1999, Paulli 1999, Zinzani 2001, Van Besien 2001, Zinzani 2002), the patients more often correspond to females in IV decade of life. Symptoms and signs are usually due to the presence of a huge (bulky) mediastinal mass; a *superior vena cava* syndrome is detected in 25-30% of subjects. The tumour shows a good response to therapy, with special reference to the MACOP-B regimen followed by radiotherapy and the HDS scheme, with includes peripheral blood stem cell transplantation. Roughly, 80% of patients achieve complete remission and 60% are alive at a 5-year follow-up. In case of relapse, the process involves the CNS, gonads, liver, kidney, pancreas, or lung.

At light microscopy (Van Besien 2001, Jaffe 2001), PMBCL usually consists of large cells with variable nuclear contours, finely dispersed chromatin, medium-sized nucleoli, at times adjacent to the nuclear membrane, and relatively wide rim of clear-acidophilic cytoplasm. Mitotic figures are numerous. The lymphomatous growth more often evokes a fibrotic reaction with compartmentalisation: especially in small biopsies, which can show filamentous degeneration of the nuclei, this might hinder the differentiation of PMBL from Hodgkin's lymphoma or non-lymphoid tumours, such as clear-cell thymoma and seminoma/dysgerminoma.

So far, phenotypic analysis has revealed positivity of PMBL for CD45 and CD20, but negativity for CD3 and a variety of other T-cell markers; CD79a has generally been detected, in spite of the usual lack of surface and cytoplasmic Ig (Harris 1994, Jaffe 2001, Addis & Isaacson 1986, Menestrina 1986, Isaacson 1987, Al-Sharabati 1991, Eichelmann 1992, Lavabre-Bertrand 1992, Falini 1995, Kanavaros 1995, Van Besien 2001). In two reported series, CD30 staining was observed in the vast majority of cases, although its was weaker and less homogeneous than in classic Hodgkin's lymphoma (cHL) and anaplastic large cell lymphoma (ALCL) (Falini 1995, Higgins & Warnke 1999). CD21 and class I and/or II histocompatibility molecules have been claimed to be absent (Addis & Isaacson 1986, Menestrina 1986, Möller 1987, Lamarre 1989). Bcl-2 protein seems to be generally expressed (Van Besien 2001), while fragmentary data are available concerning the occurrence of some molecules, such as CD10, MUM1/IRF4, PAX5/BSAP (B-cell Specific Activating Protein), Bcl-6, BOB.1, and Oct-2 (Pileri 2000, de Laval 2001, Palanisamy 2002).

In contrast to diffuse large B-cell lymphoma (DLBCL) (Aisenberg 1988, Pileri 2000), molecular studies of small series of PBCLs have so far revealed usual absence of *BCL-6* and *BCL-2* rearrangements/ mutations (Tsang 1996, Capello 2000), as well as frequent over-expression of the *MAL* gene (Copie-Bergman 1999). The latter is located on the long arm of chromosome 2 (Alonso 1998) and encodes a protein thought to play a relevant role in membrane trafficking and signaling mediated by specialized, glycolipid- and cholesterol-enriched, membrane microdomains referred as lipid rafts (Millàn 1998, Alonso 2001, Millàn 2002), which might contribute to the pathogenesis (Copie-Bergman 1999). Mutations of the gene have not been identified and the mechanism of over-expression remains unclear yet (Copie-Bergman 1999). Other reported oncogene abnormalities consist in *C-MYC* mutations and occasional detection of *REL* proto-oncogene amplification or *P53* mutations (Scarpa 1991, Tsang 1996). Due to the reported lack of *BCL-6* mutations, PMBL has been thought to recognize a pre-GC cell derivation (Capello 2000, Pileri 2000, Van Besien 2001). This assumption, however, seems to contrast with a recent report showing isotype-switched Ig genes with a high load of somatic hypermutations in a small series of PMBLs (Laithäuser 2001). Recently, an IELSG study based on 137 PMBLs has provided some new phenotypic and molecular data, which can contribute to the better understanding the process (Pileri 2002). In particular, tumor cells were characterized by the following phenotype: $CD45^+$, $CD20^+$, $CD79a^+$, $PAX5/BSAP^+$, $BOB.1^+$,

Oct-2^{+}, Bcl-2^{+}, CD30^{+}, HLA-DR^{+}, MAL protein$^{+/-}$, Bcl-6$^{+/-}$, MUM1/IRF4$^{+/-}$, CD10$^{-/+}$, CD21^{-}, CD15^{-}, CD138^{-}, CD68^{-}, and CD3^{-}. The search for immunoglobulins (Ig) as well as for the corresponding m-RNA regularly provided negative results. At molecular analysis, carried out in 45 instances, more than half the cases displayed *BCL-6* gene mutations, which usually occurred along with functioning somatic IgV_H gene mutations and Bcl-6 and/or MUM1/IRF4 expression. Frequent methylation of the *MGMT* gene was also observed. This study supports the concept that PMBL is mostly derived from activated GC or post-GCCs. However, it differs from other aggressive B-cell lymphomas and classical HL in that it shows defective Ig production in spite of the presence of the Oct-2 and BOB.1 transcription factors and the lack of IgV_H gene crippling mutations.

INTRAVASCULAR LARGE B-CELL LYMPHOMA

Intravascular large B-cell lymphoma (IVBL) is a rare clinico-pathologic subtype of DLBCL, characterised by the presence of lymphomatous cells only in the lumina of the small vessels (Jaffe 2001). It has also been termed intravascular lymphomatosis, angiotropic large cell lymphoma, angio-endotheliotropic (intravascular) lymphoma, *angioendotheliomatosis proliferans systematisata*, and malignant angioendotheliomatosis (Jaffe 2001). The process tends to simultaneously occur at numerous extranodal sites, including the CNS, skin, lung, kidney, adrenal gland, and bone marrow, without a detectable leukaemic spread (Stroup 1990, Fukuchi 1996, Sanna 1997, Jaffe 2001). IVBL more often affects patients in the 6th-7th decade of life and is associated with a variety of clinical manifestations, reflecting the organs involved, such as: dementia, focal neurological symptoms, skin nodules or plaques, nephrotic syndrome, pyrexia, hypertension, breathlessness, and haematological abnormalities (Jaffe 2001). In a proportion of cases, the tumour is associated with a haemophagocytic syndrome: the latter seems to occur rather frequently among Asian patients, thus suggesting the existence of an Asian variant of IVBL (Murase 2000, Dufau 2000). The clinical course is rapidly progressive, although complete remissions have been quoted in exceptional cases (Stroup 1990, DiGiuseppe 1994). At light microscopy, neoplastic cells are large with prominent nucleoli, possibly admixed with fibrin thrombi and at times provided with anaplastic morphology (Jaffe 2001). In the lung and bone-marrow, their recognition may be difficult without the support of immunohistochemistry (Estalilla 1999). The phenotypic profile of lymphomatous cells is similar to the one of the classical forms of DLBCL, including occasional positivity for CD30 (Jaffe 2001, Yegappan 2001). However, some specific findings have recently been proposed in the literature. CD5 and CD10 seem to be respectively more and less frequently expressed in IVBL than in classical DLBCL (Estalilla 1999, Murase 2000, Yegappan 2001). Possible myeloperoxidase positivity has been quoted by Conlin et al. (Conlin 2001). Conflicting results have been provided concerning the expression of adhesion molecules, which might explain the reason by which the tumour remains confined to the blood vessels: in fact, while Kanda et al. (1999) have reported positivity of the lymphomatous and endothelial cells for CD11a/CD49a and CD54/CD106 respectively, Ponzoni et al. (2000) have shown regular lack of CD29 and CD54. Molecular analysis reveals neither BCL-2 gene rearrangements nor EBV integration (Yegappan 2001). IgV_H gene evaluation displays that most IVBLs stem from post-GCCs, based on the presence of somatic mutation, although some heterogeneous cases may be observed (Kanda 2001).

PRIMARY EFFUSION LYMPHOMA

Primary effusion lymphoma (PEL) is a neoplasm of large B-cells usually presenting as serous effusions without detectable tumour masses (Jaffe 2001). It is regularly associated with HHV-

8/KSHV infection, EBV co-infection being detected in most instances by *in situ* hybridization (ISH), but not by immunohistochemistry for LMP-1 (Ansari 1996). PEL is usually observed in young-middle aged homosexual HIV-positive males, although sporadic cases have also been recorded in patients who underwent organ transplantation or in elderly males from areas with high prevalence of HHV-8/KSHV infection (Jaffe 2001). The tumour typically affects one cavity (pleural, peritoneal or pericardial). Other sites of involvement include the gastro-intestinal tract, soft tissue and other extranodal sites (Jaffe 2001, Boulanger 2001). Neither lymphadenopathy nor organomegaly is found. Kaposi sarcoma or multicentric Castleman disease can occasionally pre-exist (Ansari 1996, Ascoli 2001). High IL-6 and IL-10 levels can be detected in the effusions (Aoki 2000). The clinical course is highly aggressive with a median survival lower than 6 months (Jaffe 2001). Microscopic examination of cytocentrifuge preparations shows a highly pleomorphic large cell population (with co-existing immunoblastic, plasmablastic, anaplastic and Reed-Sternberg-like features), characterised by prominent nucleoli and a deeply basophilic cytoplasm, at times displaying vacuoles and/or a perinuclear hof. In routine sections, lymphomatous cells appear a bit less pleomorphic and can adhere to the pleura with focal invasion, but without the production of a tumoral mass as seen in pyothorax-associated lymphoma (see below). The phenotypic profile is thought to be quite distinctive by showing positivity for CD45, HLA-DR, EMA, CD30, CD38, and CD138, in the absence of B- and T-cell markers, as well as of surface and cytoplasmic Ig (Jaffe 2001). Carbone et al. (Carbone 2000), however, found that the comparison of IRF4 expression with that of other histogenetic markers defined two phenotypic variants of PEL, i.e. $IRF4^+$, $CD138^+$, B-cell antigens$^-$ and $IRF4^+$, $CD138^-$, B-cell antigens$^+$, suggesting a certain degree of heterogeneity in the disease histogenesis. The implications of these data are threefold. First, IRF4 expression suggests that PEL originates from post-GC, pre-terminally differentiated B-cells. Second, IRF4 may help in the differential diagnosis of PEL among other lymphomas involving the serous body cavities. Finally, IRF4 may interact with HHV-8/KSHV-encoded interferon regulatory factors (IRFs) and thus contribute to PEL escape from interferon-mediated control of viral infection. Cytogenetic studies have shown that +7, +12 and aberrations in the proximal long arm of chromosome 1 (1q) are recurring cytogenetic aberrations in PEL and have also identified breakpoints at 3q23, 7p22, 7q22, 10q24, 12q24, 13q22, 14q24, 14q32, 15p11.2 and Xq22 as well as +8, +15, +19, +X and -Y as recurring chromosome abnormalities (Wilson 2002). IgVH genes appear to be clonally rearranged and somatically mutated (Matolcsy 1998). The latter finding is in keeping with the IRF4 positivity mentioned above (Carbone 2000).

PYOTHORAX-ASSOCIATED LYMPHOMA

This condition shows some similarities with the previous one, but cannot regarded as coincidental. It characteristically occurs in patients who developed chronic pyothorax following therapeutic pneumothorax in the course of tuberculosis. It was originally described among Japanese people (Iuchi 1987), but subsequently shown to occur also in Western Countries (Ascani 1997). On clinical grounds, pyothorax-associated lymphoma (PAL) differs from PEL because of both the immunocompetence of the patient and the existence of a huge mass, which involves the pleura, thorax wall and lung. The clinical course is aggressive. At microscopic examination, it is characterised by immunoblastic morphology. Its phenotype shows complete expression of B-cell associated antigens, along with CD45, Bcl-2 protein and cytoplasmic Ig. Recent data suggest high NSE level expression both at cell level and in the serum (Nakatsuka 2002). Molecular studies reveal clonal rearrangements of IgV_H genes, along with almost regular integration of the Epstein-Barr virus at ISH (Fukayama 1993, Osawa 1995). The latter explain the

positivities observed at the search for LMP-1 and EBNA2 . The role of EBV in the promotion of the tumour remains uncertain: the production of an immunosuppressive cytokine from PAL cells, human histocompatibility leukocyte antigen class I alleles of patients with PAL, and the mutations of cytotoxic T lymphocyte epitopes in an EBV-latent antigen might have much more relevance than EBV infection *per se* (Kanno & Aozasa 1998). Analogously to PEL, a recent study by IS-PCR and TaqMan PCR has shown that pyothorax-associated lymphoma may at times be associated with HHV-8/KSHV infection (O'Donovan 2001). In the same study, HHV-8 v-IL-6, v-cyclin, and GPCR encoded transcripts were identified using RNA TaqMan PCR, v-IL-6 being significantly high. The possible presence of HHV-8 in PAL raises interesting questions in relation to the pathobiology of the condition. Although HHV-8 cannot be regarded as an obligate pathogen, necessary for the effusion phenotype, it might contribute to tumour development and progression by its secretion of specific cytokines (O'Donovan 2001).

REFERENCES

- Abou-Elella AA, Weisenburger DD, Vose JM, et al. Primary mediastinal large B-cell lymphoma: a clinico-pathologic study of 43 patients from the Nebraska lymphoma study group. J Clin Oncol 1999; 17:784-790.
- Achten R, Verhoef G, Vanuytsel L, et al. Histiocyte-rich, T-cell-rich B-cell lymphoma: a distinct diffuse large B-cell lymphoma subtype showing characteristic morphologic and immunophenotypic features. Histopathology 2002; 40:31-45.
- Addis BJ, Isaacson PG. Large cell lymphoma of the mediastinum: a B-cell tumor of probable thymic origin. Histopathology 1986; 10:379-390.
- Aisenberg AC, Wilkes BM, Jacobson JO. The bcl-2 gene is rearranged in many diffuse B-cell lymphomas. Blood 1988; 71:969-972.
- Akasaka T, Ueda C, Kurata M, et al. Nonimmunoglobulin (non-Ig)/BCL-6 gene fusion in diffuse large B-cell lymphoma results in worse prognosis than Ig/BCL6. Blood 2000; 96:2907-2909.
- Alizadeh AA, Eisen MB, Davis E, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature 2000; 403:503-511.
- Alonso MA, Barton DE, Francke U. Assignement of the T cell differentiation gene *MAL* to human chromosome 2, region cen-q13. Immunogenetics 1998; 27:91-95.
- Alonso MA, Millán J. The role of lipid rafts in signalling and membrane trafficking in t lymphocytes. J Cell Sci 2001; 114:3957-3965.
- Al-Sharabati M, Chittal S, Duga-Neulat I, et al. Primary anterior mediastinal B-cell lymphoma. Cancer 1991; 67:2579-2587.
- Armitage JO, Weisenburger DD. New approach to classifying non-Hodgkin's lymphomas: clinical features of the major histologic subtypes. Non Hodgkin's Lymphoma Classification Project. J Clin Oncol 1998; 16:2780-2795.
- Anon. A clinical evaluation of the International Lymphoma Study Group classification of non-Hodgkin's lymphoma. The non-Hodgkin's lymphoma classification project. Blood 1997; 89:3909-3918.
- Ansari MQ, Dawson DB, Nador R et al. Primary body cavity-based AIDS-related lymphomas. Am J Clin Pathol 1996; 105:221-229.
- Aoki Y, Yarchoan R, Braun J, et al. Viral and cellular cytokines in AIDS-related malignant lymphomatous effesions. Blood 2000; 96:1599-1601.

- Artiga MJ, Saez AI, Romero C, et al. A short mutational hot spot in the first intron of *BCL-6* is associated with increased *BCL-6* expression and with longer overall survival in large B-cell lymphomas. Am J Pathol 2002; 160:1371-1380.
- Ascani S, Piccioli M, Poggi S, et al. Pyothorax-associated lymphoma: description of the first two cases detected in Italy. Ann Oncol 1997; 8:1133-1138.
- Ascoli v, Signoretti S, Onetti-Muda A, et al. Primary effusion lymphoma in HIV-infected patients with multicentric Castleman's disease. J Pathol 2001; 193:200-209.
- Baars JW, De Jong D, Willemse EM, et al. Diffuse large B-cell non Hodgkin lymphomas: the clinical relevance of histological subclassification. Br J Cancer 1999; 79:1770-1776.
- Barrans SL, O'Connor SJ, Evans PA, et al. Rearrangement of the BCL6 locus at 3q27 is an indipendent poor prognostic factor in nodal diffuse large B-cell lymphoma. Br J Haematol 2002 (a); 117:322-332.
- Barrans SL, Carter I, Owen RG, et al. Germinal center phenotype and bcl-2 expression combined with the International Prognostic Index improves patient risk stratification in diffuse large B-cell lymphoma. Blood 2002 (b); 99:1136-1143.
- Boulanger E, Agbalika F, Maarek O, et al. A Clinical, molecular and cytogenetic study of 12 cases of human herpesvirus 8 associated primary effusion lymphoma in HIV-infected patients. Hematol J 2001; 2:172-179.
- Cabanillas F, Velasquez WS, Hagemeister FB, et al. Clinical. Biologic, and histologic features of late relapses in diffuse large cell lymphomas. Blood 1992; 79:1024-1028.
- Capello D, Vitolo U, Pasqualucci L, et al. Distribution and pattern of BCL-6 mutations throughout the spectrum of B-cell neoplasia. Blood 2000; 95:651-659.
- Carbone A, Gaidano G, Gloghini A, et al. Differential expression of Bcl-6, CD138/syndecan-1, and EBV-encoded latent membrane protein-1 identifies distinct histogenetic subsets of acquired immunodeficiency syndrome-related non-Hodgkin's lymphomas. Blood 1998; 91:747-755.
- Carbone A, Gloghini A, Cozzi MR et al. Expression of MUM1/IRF4 selectively clusters with primary effusion lymphoma among lymphomatous effusions: implications for disease histogenesis and pathogenesis. Br J Haematol 2000; 111:247-257.
- Carbone A, Gloghini A, Larocca LM, et al. Expression profile of MUM1/IRF4, BCL-6, and CD138/syndecan-1 defines novel histogenetic subsets of human immunodeficiency virus-related lymphomas. Blood 2001; 97:744-751.
- Cazals-Hatem D, Lepage E, Brice P, et al. Primary mediastinal large B-cell lymphoma: a clinicopathologic study of 141 cases compared with 916 nonmediastinal large B-cell lymphomas – A GELA ("Group d'Etude des Lymphomes de l'Adulte") study. Am J Surg Pathol 1996; 20:877-888.
- Chim CS, Liang R, Chan AC. Sclerosing malignant lymphoma mimiching idiopathic retroperitoneal fibrosis: importance of clonality study. Am J Med 2001; 111:240-241.
- Chittal SM, Brousset P, Voigt JJ, et al. Large B-cell lymphoma rich in T-cells and simulating Hodgkin's disease. Histopathology 1991; 19:211-220.
- Conlan MG, Bast M, Armitage JO, et al. Bone marrow involvement by non-Hodgkin's lymphoma: the clinical significance of morphologic discordance between the lymph node and bone marrow. Nebraska Lymphoma Study Group. J Clin Oncol 1990; 8:1163-1172.
- Conlin PA, Orden MB, Hough TR, et al. Myeloperoxidase-positive intravascular large B-cell lymphoma. Arch Pathol Lab Med 2001; 125:948-950.
- Copie-Bergman C, Gaulard P, Mauche-Chretien L, et al. The *MAL* gene is expressed in primary mediastinal large B-cell lymphoma. Blood 1999; 94:3567-3575.

- Delabie J, Vandenberghe E, Kennes C, et al. Histiocyte-rich B-cell lymphoma. A distinct clinicopathologic entity possibly related to lymphocyte predominant Hodgkin's disease, paragranuloma subtype. Am J Surg Pathol 1992; 16:37-48.
- de Laval L, Ferry JA, Falini B, et al. Expression of Bcl-6 and CD10 in primary mediastinal large B cell lymphoma: evidence for derivation from germinal center B cells? Am J Surg Pathol 2001; 25:1277-1282.
- Diebold J, Anderson JR, Armitage JO, et al. Diffuse large B-cell lymphoma: a clinicopathologic analysis of 444 cases classified according to the updated Kiel classification. Leuk Lymphoma 2002; 43:97-104.
- DiGiuseppe JA, Nelson WC, Seifter EJ, et al. Intravascular lymphomatosis: a clinicopathologic study of 10 cases and assessment of response to chemotherapy. J Clin Oncol 1994; 12:2573-2579.
- Dogan A, Badgi E, Munson P, Isaacson PG: CD10 and Bcl-6 expression in paraffin sections of normal lymphoid tissue and B-cell lymphomas. Am J Surg Pathol 2000, 24:846-52.
- Dufau JP, Le Tourneau A, Molina T, et al. Intravascular large B-cell lymphoma with bone marrow involvement at presentation and haemophagocytic syndrome: two Western cases in favour of a specific variant. Histopathology 2000; 37:509-512.
- Eichelmann A, Koretz K, Mechtersheimer G, Möller P: Adhesion receptor profile of thymic B-cell lymphoma. Am J Pathol 1992, 141:729-741.
- Engelhard M, Brittinger G, Huhn D, et al. Subclassification of diffuse large B-cell lymphomas according to the Kiel classification: distinction of centroblastic and immunoblastic lymphomas is a significant prognostic risk factor. Blood 1997; 89:2291-2297.
- Eshoa C, Perkins S, Kampalath B, et al. Decreased CD10 expression in grade III and in interfollicular infiltrates of follicular lymphomas. Am J Clin Pathol 2001; 115:862-867.
- Estalilla OC, Koo CH, Brynes RK, et al. Intravascular large B-cell lymphoma. A report of five cases initially diagnosed by bone marrow biopsy. Am J Clin Pathol 1999; 112:248-255.
- Esteller M, Gaidano G, Goodman SN, et al. Hypermethylation of the DNA repair gene O(6)-methylguanine DNA methyltransferase and survival of patients with diffuse large B-cell lymphoma. J Natl Cancer Inst 2002; 94:26-32.
- Falini B, Pileri S, Stein H, et al. Variable expression of leukocyte common antigen (CD45) in CD30 (Ki-1)-positive anaplastic large cell (ALC) lymphoma: implication for the differential diagnosis between lymphoid and non lymphoid malignancies. Hum Pathol 1990; 21:624-629.
- Falini B, Venturi S, Martelli M, et al. Mediastinal large B-cell lymphoma: clinical and immuno-histochemical findings of 18 patients treated with two different third generation regimens. Br J Haematol 1995; 89:780-789.
- Falini B, Fizzotti M, Pucciarini A, et al. A monoclonal antibody (MUM1p) detects expression of the MUM1/IRF-4 protein in a subset of germinal center B cells, plasma cells and activated T-cells. Blood 2000; 95:2084-2092.
- Falini B, Mason DY. Proteins encoded by genes involved in chromosomal alterations in lymphoma and leukemia: clinical value of their detection by immunocytochemistry. Blood 2002; 99(2):409-426.
- Filipits M, Jaeger U, Pohl G, et al. Cyclin D3 is a predictive and prognostic factor in diffuse large B-ell lymphoma. Clin Cancer Res 2002; 8:729-733.

- Fisher RI, Jones RB, DeVita VT Jr, et al. Natural history of malignant lymphomas with divergent histologies at staging evaluation. Cancer 1981; 47:2022-2025.
- Flaitz CM, Nichols CM, Walling DM, Hicks MJ. Plasmablastic lymphoma: an HIV-associated entity with primary oral manifestations. Oral Oncol 2002; 38:96-102.
- Fukayama M, Ibuka T, Hayaschi Y, et al. Epstein-Barr virus in pythorax-associated pleural lymphoma. Am J Pathol 1993; 143:1044-1049.
- Fukuchi M, Fushimi S, Yoneya M, et al. An autopsy case of intravascular malignant lymphoma presenting with intracranial B-cell type malignant lymphoma. Noshuyo Byori 1996; 13:119-125.
- Gascoyne RD, Adomat SA, Krajewski S, et al. Prognostic significance of Bcl-2 protein expression and *BCL-2* gene rearrangement in diffuse aggressive non-Hodgkin's lymphoma. Blood 1997; 90:244-251.
- Guinee D Jr, Jaffe ES, Kingma D, et al. Pulmonary lymphomatoid granulomatosis. Evidence for a proliferation of Epstein-Barr virus infected B-lymphocytes with a prominent T-cell component and vasculitis. Am J Surg Pathol 1994; 18:753-764.
- Harris NL, Jaffe ES, Stein H, et al. A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. Blood 1994; 84:1361-1392.
- Higgins JP, Warnke RA. CD30 expression is common in mediastinal large B-cell lymphoma. Am J Clin Pathol 1999; 112:241-247.
- Hill ME, MacLennan KA, Cunningham DC, et al. Prognostic significance of *BCL-2* expression and bcl-2 major breakpoint region rearrangement in diffuse large cell non-Hodgkin's lymphoma: a British National Lymphoma Investigation Study. Blood 1996; 88:1046-1051.
- Hodges GF, Lenhardt TM, Cotelingam JD. Bone marrow involvement in large-cell lymphoma. Prognostic implication of discordant disease. Am J Clin Pathol 1994; 101:305-311.
- Huang JZ, Sanger WG, Greiner TC, et al. The t(14;18) defines a unique subset of diffuse large B-cell lymphoma with a germinal center B-cell gene expression profile. Blood 2002; 99:2285-2290.
- Isaacson PG, Norton AJ, Addis BJ: The human thymus contains a novel population of B lymphocytes. Lancet 1987; 2:1488-1491.
- Iuchi K, Ichimiya A, Akaschi A, et al. Non-Hodgkin's lymphoma of the pleural cavity developing from long-standing pyothorax. Cancer 1987; 60:1771-1775.
- Jaffe ES, Harris NL, Stein H, Vardiman JW. Tumours of haematopoietic and lymphoid tissues. IARC Press, International Agency for Research on Cancer, Lyon 2001; 175-176.
- Jerkeman M, Johansson B, Akerman H, et al. Prognostic implications of cytogenetic aberrations in diffuse large B-cell lymphomas. Eur J Haematol 1999; 62:184-190.
- Joos S, Otano-Joos MI, Ziegler S, et al. Primary mediastinal (thymic) B-cell lymphoma is characterized by gains of chromosomal material including 9p and amplification of the *REL* gene. Blood 1996; 87:1571-1578.
- Jundt F, Kley K, Anagnostopoulos I, et al. Loss of PU.1 expression is associated with defective immunoglobulin transcription in Hodgkin and Reed-Sternberg cells of classical Hodgkin disease. Blood 2002; 99:3060-3062.
- Kanavaros P, Gaulard P, Charlotte F, et al. Discordant expression of immunoglobulin and its associated molecule mb-1/CD79a is frequently found in mediastinal large B cell lymphomas. Am J Pathol 1995; 146:735-741.

- Kanda M, Suzumiya J, Ohshima K, et al. Intravascular large cell lymphoma: clinicopathological, immunohistochemical and molecular genetic studies. Leuk Lymphoma 1999; 34:569-580.
- Kanda M, Suzumiya J, Ohshima K, et al. Analysis of the immunoglobulin heavy chain gene variable region of intravascular large B-cell lymphoma. Virchows Arch 2001; 439:540-546.
- Kanno H, Aozasa K. Mechanism for development of pyothorax-associated lymphoma. Pathol Int 1998; 48:653-664.
- Kramer MH, Hermans J, Wijburg E, et al. Clinical relevance of *BCL-2*, *BCL-6*, and *MYC* rearrangements in diffuse large B-cell lymphoma. Blood 1998; 92:3152-3162.
- Kuttler F, Valnet-Rabier MB, Angonin R, et al. Relationship between expression of genes involved in cell cycle control and apoptosis in diffuse large B cell lymphoma: a preferential survivin-cyclin B link. Leukemia 2002; 16:726-735.
- Laithäuser F, Bäuerle M, Huynh MQ, Möller P: Isotype-switched immunoglobulin genes with a high load of somatic hypermutation and lack of ongoing mutational activity are prevalent in mediastinal B-cell lymphoma. Blood 2001; 98:2762-2770.
- Lamarre L, Jacobson JO, Aisenberg AC, Harris NL: Primary large cell lymphoma of the mediastinum: a histologic and immunophenotypic study of 29 cases. Am J Surg Pathol 1989; 13:730-739.
- Lavabre-Bertrand T, Donadio D, Fegueux N, et al. A study of 15 cases of primary mediastinal lymphoma of B-cell type. Cancer 1992; 50:2561-2566.
- Lazzarino M, Orlandi E, Paulli M, et al. Primary mediastinal B-cell lymphoma with sclerosis: an aggressive tumor with distinctive clinical and pathologic features. J Clin Oncol 1993; 11:2306-2313.
- Lennert K, Feller AC. Histopathology of non-Hodgkin's lymphomas. Berlin: Springer-Verlag. 1992.
- Lichtestein A, Levine A, Taylor C, et al. Primary mediastinal lymphoma in adults. Am J Med 1980; 68:509-514.
- Lo Coco F, Ye BH, Lista F, et al. Rearrangements of the *BCL-6* gene in diffuse large cell non-Hodgkin's lymphoma. Blood 1994; 83:1757-1759.
- Lossos IS, Alizadeh AA, Eisen MB, et al. Ongoing immunoglobulin somatic mutation in germinal center B cell-like but not in activated B cell-like diffuse large cell lymphomas. Proc Natl Acad Sci USA 2000; 97:10209-10213.
- Lossos IS, Jones CD, Warnke R, et al. Expression of a single gene, BCL-6, strongly predicts survival in patients with diffuse large B-cell lymphoma. Blood 2001; 98:945-951.
- Matolcsy A, Nador RG, Cesarman E, Knowles DM. Immunoglobulin VH gene mutational analysis suggests that primary effusion lymphomas derive from different stages of B cell maturation. Am J Pathol 1998; 153:1609-1614.
- Menestrina F, Chilosi M, Bonetti F, et al. Mediastinal large-cell lymphoma of B-type, with sclerosis: histopathological and immunohistochemical study of eight cases. Histopathology 1986; 10:589-600.
- Millán J, Alonso MA: MAL, a novel integral membrane protein of human T lymphocytes, associates with glycosylphosphatidylinositol-anchored proteins and Src-like tyrosine kinases. Eur J.Immunol 1998, 28:3675-3684.
- Millán J, Montoya MC, Sancho D, Sánchez-Madrid F, Alonso, MA: Lipid rafts mediate biosynthetic transport to the T cell uropod subdomain and are necessary for uropod integrity and function. Blood 2002; 99:978-984.

- Möller P, Moldenhauer G, Momburg F, et al. Mediastinal lymphoma of clear cell type is tumor corresponding to terminal steps of B-cell differentiation. Blood 1987; 59:1087-1095.
- Murase T, Nakamura S, Kawauchi K, et al. An Asian variant of intravascular large B-cell lymphoma: clinical, pathological and cytogenetic approaches to diffuse large B-cell lymphoma associated with haemophagocytic syndrome. Br J Haematol 2000; 111:826-834.
- Natkunam Y, Warnke RA, Montgomery K, et al. Analysis of MUM1/IRF4 protein expression using tissue microarrays and immunohistochemistry. Mod Pathol 2001; 14:686-694.
- Nakatsuka S, Nishiu M, Tomita Y, et al. Enhanced Expression of Neuron-specific Enolase (NSE) in Pyothorax-associated Lymphoma (PAL). Jpn J Cancer Res 2002; 93:411-416.
- Nozawa Y, Wang J, Weiss LM, et al. Diffuse large B-cell lymphoma with spindle cell features. Histopathology 2001; 38:177-178.
- O'Donovan M, Silva I, Uhlmann V, et al. Expression profile of human herpesvirus 8 (HHV-8) in pyothorax associated lymphoma and in effusion lymphoma. Mol Pathol 2001; 54:80-85.
- Offit K, Lo Coco F, Louie DC, et al. Rearrangement of the *BCL-6* gene as a prognostic marker in diffuse large-cell lymphoma. N Engl J Med 1994; 331:74-80.
- Ohsawa M, Tomita Y, Kanno H, et al. Role of Epstein-Barr virus in pleural lymphomagenesis. Mod Pathol 1995; 8:848-853.
- Ohshima K, Kawasaki C, Muta H, et al. CD10 and Bcl10 expression in diffuse large B-cell lymphoma: CD10 is a marker of improved prognosis. Histopathology 2001; 39:156-162.
- Palanisamy N, Abou-Elella AA, Chaganti SR, et al. Similar patterns of genomic altrations characterize primary mediastinal large-B-cell lymphoma and diffuse large-B-cell lymphoma. Genes Chromosomes Cancer 2002; 33:114-122.
- Paulli M, Strater J, Gianelli U, et al. Mediastinal B-cell lymphoma: a study of its histomorphologic spectrum based on 109 cases. Hum Pathol 1999; 30:178-187.
- Perrone T, Frizzera G, Rosai J. Mediastinal diffuse large-cell lymphoma with sclerosis: a clinicopathologic study of 60 cases. Am J Surg Pathol 1986; 10:176-191.
- Pileri SA, Ascani S, Sabattini E, et al. The pathologist's view point: Part II - aggressive lymphomas. Haematologica 2000; 85:1285-1298.
- Pileri SA, Zinzani PL, Ascani S, et al. Primary retroperitoneal large B-cell lymphoma. Clinico-pathologic study of 9 cases. Ann Oncol 2001; 12:1445-1453.
- Pileri SA, Gaidano G, Zinzani PL, et al. Primary mediastinal B-cell lymphoma (PMBL): high frequency of *BCL-6* mutations and preserved transcriptional activity in the absence of immunoglobulin expression. A co-operative study of the International Extranodal Lymphoma Study Group. Am J Pathol 2002 (a), in press.
- Pileri SA, Ascani S, Leoncini L, et al. Hodgkin's lymphoma: the pathologist view point. J Clin Pathol 2002 (b); 55:162-176.
- Ponzoni M, Arrigoni G, Gould VE, et al. Lack of CD29 (beta1 integrin) and CD54 (ICAM-1) adhesion molecules in intravascular lymphomatosis. Hum Pathol 2000; 31:220-226.
- Ramsay AD, Smith WJ, Isaacson PG, et al. T-cell-rich B-cell lymphoma. Am J Surg Pathol 1988; 12:433-443.

- Ree HJ, Yang WI, Kim CW, et al. Coexpression of Bcl-6 and CD10 in diffuse large B-cell lymphomas: significance of Bcl-6 expression patterns in identifying germinal center B-cell lymphoma. Hum Pathol 2001; 32:954-962.
- Riguad G, Moore PS, Taruscio D, Scardoni M, Montresor M, Menestrina F, Scarpa A: Alterations of chromosome arm 6p is characteristic of primary mediastinal B-cell lymphoma, as identified by genome-wide allelotyping. Genes Chromosomes Cancer 2001; 31:191-195.
- Robertson LE, Redman JR, Butler JJ, et al. Discordant bone marrow involvement in diffuse large-cell lymphoma: a distinct clinical-pathologic entity associated with a continuous risk of relapse. J Clin Oncol 1991; 9:236-242.
- Sanna P, Bertoni F, Roggero E, et al. Angiotropic (intravascular) large cell lymphoma: case report and short discussion of the literature. Tumori 1997; 83:772-775.
- Scarpa A, Bonetti F, Menestrina F, Chilosi M, Lestani M, Bovolenta C, Zamnobi G, Fiore-Donati L: Mediastinal large cell lymphoma with sclerosis: genotypic analysis establishes its B nature. Virchows Arch A Pathol Anat Histopathol 1987; 412:17-21.
- Scarpa A, Borgato L, Chilosi M, et al. Evidence of *c-MYC* gene abnormalities in mediastinal large B-cell lymphoma of young adult age. Blood 1991; 78:780-788.
- Shipp MA, Ross KN, Tamayo P, et al. Diffuse large B-cell lymphoma outcome prediction by gene-expression profiling and supervised machine learning. Nat Med 2002; 8:68-74
- Stroup RM, Sheibani K, Moncada A, et al. Angiotropic (intravascular) large cell lymphoma. A clinicopathologic study of seven cases with unique clinical presentations. Cancer 1990; 66:1781-1788.
- Torlakovic E, Tierens A, Dang HD, Delabie J. The transcription factor PU.1, necessary for B-cell development is expressed in lymphocyte predominance, but not classical Hodgkin's disease. Am J Pathol 2001; 159(5):1807-1814.
- Tsang P, Cesarman E, Chadburn A, Liu YF, Knowles DM: Molecular characterization of primary mediastinal B cell lymphoma. Am J Pathol 1996; 148:2017-2025.
- Van Besien K, Kelta M, Bahaguna P: Primary mediastinal B-cell lymphoma: a review of pathology and management. J Clin Oncol 2001; 19:1855-1864.
- Vitolo U, Gaidano G, Botto B, et al. Rearrangements of *BCL-6*, *BCL-2*, *c-MYC* and 6q deletion in B-diffuse large-cell lymphoma: clinical relevance in 71 patients. Ann Oncol 1998; 9:55-61.
- Vitolo U, Botto B, Capello D, et al. Point mutations of the *BCL-6* gene: clinical and prognostic correlation in B-diffuse large cell lymphoma. Leukemia 2002; 16:268-275.
- Wilson KS, Mckenna RW, Kroft SH, et al. Primary effusion lymphomas exhibit complex and recurrent cytogenetic abnormalities. Br J Haematol 2002; 116:113-121.
- Xu Y, McKenna RW, Molberg KH, et al. Clinicopathologic analysis of CD10+ and CD10- diffuse large B-cell lymphoma. Identification of a high-risk subset with coexpression of CD10 and bcl-2. Am J Clin Pathol 2001; 116:183-190.
- Yamaguchi M, Seto M, Okamoto M, et al. De novo CD5+ diffuse large B-cell lymphoma: a clinicopathologic study of 109 patients. Blood 2002; 99:815-821.
- Ye BH, Lista F, Lo Coco F, et al. Alterations of a zinc finger-encoding gene, *BCL-6*, in diffuse large-cell lymphoma. Science 1993; 262:747-750.
- Yegappan S, Coupland R, Arber DA, et al. Angiotropic lymphoma: an immunophenotypically and clinically heterogeneous lymphoma. Mod Pathol 2001; 14:1147-1156.

- Zinzani PL, Martelli M, Bertini M, et al. Induction chemotherpy strategies for primary mediastinal large B-cell lymphoma with sclerosis: a retrospective multinational study on 426 previously untreated patients. Br J Haematol 2002, in press.
- Zinzani PL, Martelli M, Bertini M, et al. Primary mediastinal large B-cell lymphoma with sclerosis: a clinical study of 89 patients with MACOP-B chemotherapy and radiation therapy. Haematologica 2001; 86:187-191.

Table 1. Morphologic subtypes of DLBCL

Centroblastic

Immunoblastic

Anaplastic

Plasmablastic

Multilobated

T-cell-rich/Histiocyte-rich B-cell lymphoma

With spindle cell features

Table 2. Criteria for the differential diagnosis between T-cell-rich B-cell lymphoma (TCRBCL) and lymphocyte predominant (LP-HL) or classical Hodgkin's lymphoma (CHL).

Diagnostic criteria	**TCRBCL**	**LP-HL nodular/diffuse**	**CHL**
Neoplastic component			
Cell distribution	Dispersed	Within the nodules	Dispersed
L&H/L&H-like cells	-/+	+/-	-
RS/RS-like cells	-/+	-/+	-
CD45	+	+	-
CD30	-*	-*	+
CD15	-	-	+
CD79a	+/-	-/+	-/+ Variable
CD20	+**	+**	-/+ Variable
BOB.1	+	+	-
Oct2	+***	+***	Rare
PU1	+	+	-
Bcl-6 protein	+	+	-
CD3	-	-	-/+
EMA	+/-	+/-	Rare
J-Chain	+/-	+/-	-
Mib-1/Ki-67	High	High	High
EBV (LMP-1/EBER)	-	-	+ Variable
VH Ig gene rearrangement	+	+	+#
Reactive component			
T-lymphocytes	Numerous	Moderate	Variable
T-lymphocytes with irregular nuclear profile	+/-	-	-/+
Bcl-6$^+$/CD57$^+$ rosettes	-	Numerous	-
Amount of TIA-1$^+$ cells	Very high	Low	High
Amount of CD20$^+$ small lymphocytes	Low	High	Low
Histiocytes	Variable	Some	Variable
FDC	-	+	+
Clinical findings			
Stage	III/IV	I/II	I/III
Bone-marrow involvement	+/-	-	-
Orderly progression in the spread	-	-	+

Abbreviations

FDC: follicular dendritic cells
*: weakly positive in some instances **: negative in rare instances ***: usually overexpressed
#: in 1-2% of the cases TCR gene rearrangement

Table 3. Clinical subtypes of DLBCL

Primary mediastinal large B-cell lymphoma (PMBCL)

Intravascular large B-cell lymphoma (IV)

Primary effusion lymphoma (PEL)

Pyothorax-associated pleural lymphoma (PAL)

QUESTIONS

1. An updated and useful subclassification of diffuse large B-cell lymphomas should be based on:
   - **A.** Cell morphology.
   - **B.** Clinical indicators.
   - **C.** Phenotype.
   - **D.** Phenotypic and molecular findings.
2. Primary mediastinal large B-cell lymphoma is derived from:
   - **A.** A virgin B-cell.
   - **B.** A germinal centre or post-germinal centre B-cell.
   - **C.** A B-cell precursor.
   - **D.** A not yet well-defined B-cell compartment.

***Mark the appropriate letter.**

1. La sottoclassificazione di maggiore utilità nei linfomi a grandi cellule B di tipo diffuso è quella:
   - **A.** Morfologica.
   - **B.** Clinica.
   - **C.** Fenotipica.
   - **D.** Fenotipica e molecolare.
2. Il linfoma a grandi cellule B primitivo del mediastino più spesso deriva da:
   - **A.** Cellule B vergini.
   - **B.** Elementi del centro germinativo o post-centro germinativo.
   - **C.** Precursori del sistema B linfocitario.
   - **D.** Da una cellula B ancora non meglio identificata.

***Contrassegnare la lettera giusta.**

THE ROLE OF ELECTRON MICROSCOPY IN THE DIAGNOSIS OF SOFT-TISSUE TUMOURS.

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INTRODUCTION

Electron microscopy is one of several investigative techniques for diagnosing soft-tissue lesions:

clinical findings and radiology
histology, using classical stains
immunohistochemistry
electron microscopy
cytogenetics
molecular genetics

Many investigators regard electron microscopy applied to tumour diagnosis as old-fashioned and unnecessary, not only in the context of the more fashionable and seemingly more promising molecular genetics techniques, but also from the point of view of financial cost-effectiveness. However, it is a matter of personal experience as well as being documented in the literature (Kandel et al 1998; Mierau 1999; Eyden 1999, 2002; Lloreta-Trull et al 2000; Tucker 2000) that there continue to be encountered tumours whose diagnosis is confirmed, refined or corrected using electron microscopy. It is therefore a technique which is still important today, even though there may be a question mark about its role in, perhaps, 10 or 20 years time. Having said that, the diagnostic need for electron microscopy in a given institution is very largely dictated by the philosophy and requirements of the pathology department director. For the entire period in which there has been a diagnostic electron microscopy facility at the Christie Hospital – the largest specialist cancer centre in the UK - the Directors of Pathology have consistently taken the view that, within reasonable contraints of time and money, all possible avenues of investigation should be utilised to investigate tumours, especially soft-tissue tumours and lesions. This approach is one which maximises diagnostic confidence, even though some of the investigations contributing to a final confidently held diagnosis may not in themselves alter patient management. Closely linked to this philosophy is the need to maintain academic research, where valuable and interesting insights into tumour cell differentiation can also be gained from electron microscopy, even though they may be outside the purely diagnostic arena.

Soft-tissue tumours present difficulties for pathologists for a number of reasons, but one main reason is that relative to carcinomas, for example, they are uncommon. Consequently, it may be difficult to gain the wide experience needed for diagnostic

confidence outside large or specialised centres. Partly because of their infrequent occurrence, they present opportunities for diagnostic confusion with the other main groups of tumours like melanoma and
carcinoma. In this exercise, the pathologist can make use of electron microscopy because the main groups of soft-tissue lesions have distinctive ultrastructural features, as follows:

Tumour type*	***distinctive organelles***
Fibrous/cartilaginous/ bone	rough endoplasmic reticulum
Fibrohistiocytic	rough endoplasmic reticulum, lysosomes
Peripheral nerve sheath	lamina and processes
Neuronal	neuroendocrine granules processes with intermediate filaments or microtubules
	synapses
Smooth-muscle	myofilaments with focal densities attachment plaques and lamina
Striated muscle	geometric arrays of myofilaments
Vascular	lumina, Weibel-Palade bodies
Lipomatous	lipid
	...
compare with ...	
melanoma	melanosomes
carcinoma	desmosomes; tonofibrils; lumina

* classification loosely based on Enzinger 4th edition

However, there are many instances where genetic abnormalities intervene to give rise to loss of anticipated features or the appearance of unexpected ones,
compared with the putative normal cell counterpart. This can apply to all diagnostic investigations, not just electron microscopy. In the field of immunohistochemistry, for

example, one often talks of *aberrant or anomalous immunostaining* for such unanticipated features. Malignant tumours especially tend to lose features.

This talk will focus on examples of problematic tumours taken from the files of the Christie Hospital, illustrating something of the range of applications of electron microscopy in the diagnosis of soft-tissue lesions and emphasising the continuing role of electron microscopy in contemporary tumour diagnosis.

Before proceeding to these examples, however, it is appropriate to mention a few salient points about technique and organisation.

ORGANISATIONAL AND TECHNICAL ASPECTS OF MAXIMISING DIAGNOSTIC INFORMATION

Wherever possible, tissue should be fixed fresh in a cacodylate- or phosphate-buffered glutaraldehyde. Failing that, wide sampling (for example, ten millimetre-cube pieces of tissue) from different areas of the specimen in histological formalin should provide a means of encountering the better preserved areas. Retrieval from wax should only be used as a last resort, but preservation can be good enough for interpretation, and probably depends more on the initial quality of fixation than the severe conditions of the wax-embedding procedure.

Extensive sectioning at the 1-2 □m level with staining in toluidine-blue is desirable to confirm a histology consistent with that seen in the H&E section, but also to locate the better preserved areas. Well preserved cells have pale nuclei with little peripheral nuclear chromatin, while poorly preserved cells have clear nuclear interiors and a well defined nuclear periphery, as chromatin artifactually condenses on to the inner surface of the nucleus and soluble substances are leached out. Cytoplasmic organelles can differ significantly in well and poorly preserved cells, and the selection of the better preserved areas may be influential in the diagnostic process.

In the examination of ultrathin sections one needs to avoid misinterpreting reactive elements: indeed, any well differentiated cell in a malignant tumour especially should be at least suspected of being an entrapped or a reactive cell. One important pitfall, which can be seen in the literature, involves vessels with closed lumina, in which the lamina of endothelium has been misinterpreted as a marker for muscle or nerve sheath tumour cell differentiation (Sawada et al 1992; Pantuck et al 1996).

APPLICATIONS

It is important to emphasise that electron microscopy provides information on phenotype (cellular differentiation) and it is not very good at distinguishing between benign and malignant tumours, except when it contributes to identifying a given entity with a known clinical behaviour.

Case 1 illustrates the difficulty of electron microscopy in distinguishing between a benign and a malignant tumour. A 60-year old woman had a spinal tumour in which the histology and immunohistochemistry indicated a differential of cellular Schwannoma versus malignant peripheral nerve sheath tumour. Ultrastructure revealed lamina coating whole cells and cell processes, as well as irregular nuclei. The cell population was monomorphic

and this was the only clue favouring a malignancy rather than a more benign lesion, on the basis that benign Schwannian tumours often include non-Schwannian cells like fibroblasts and perineurial cells in a heterogeneous cell population. This diagnosis of a malignant peripheral nerve sheath tumour, however, was a very tentative conclusion and reflects the fact that electron microscopy is not primarily useful in distinguishing benign from malignant tumours.

It is essential in the histopathological diagnosis of soft-tissue lesions to distinguish between a sarcoma (or mesenchymal tumour), on the one hand, and a melanoma or carcinoma, on the other, because of differences in the management of these broad groups of tumours.

Case 2 is of a lesion on the forehead of a 76 year old man which showed a pleomorphic/spindled cell morphology and a lack of immunoreactivity, apart from vimentin. The differential included malignant melanoma, spindle cell carcinoma, true sarcoma, and atypical fibroxanthoma. The negative immunostainings favoured atypical fibroxanthoma and this was confirmed partly by the negative features of an absence of melanosomes, tonofibrils and desmosomes, but also by the presence of the clear vacuoles characteristic of atypical fibroxanthoma.

Case 3 illustrated a problem case on the scalp of a 41 year old man where the differential was between a PNET and an amelanotic malignant melanoma. By electron microscopy no melanosomes were seen, but unambiguous examples of neuroendocrine granules were encountered, confirming neuroendocrine or neuronal differentiation, and indicating, in the present context, PNET.

The foregoing cases have illustrated cases involving malignant melanoma, PNET and a mesenchymal tumour: the following three cases illustrate difficulties in distinguishing between sarcoma/mesenchymal tumour and carcinoma.

Case 4 was a round-cell/epithelioid tumour with abundant hyalinisation between individual cells and groups of cells. It was referred as a fibromatosis but carcinoma was also strongly considered although malignant melanoma and lymphoma also entered the differential. There was no positive immunostaining except for vimentin. By electron microscopy, prominent rough endoplasmic reticulum (rER) was found, but the myofilaments which would have been required to confirm a fibromatosis were absent. There was a prominent Golgi apparatus producing collagen secretion granules, and this, in combination with rER, suggested a matrix-producing mesenchymal tumour. In the absence of cartilaginous or osseous differentiation, the tumour was diagnosed as a sclerosing epithelioid fibrosarcoma.

In contrast to the foregoing case where several diagnoses were considered in a non-immunoreactive context, the next case showed positive immunoreactivity for several markers, indicating different (divergent) cell differentiations.

Case 5 was an osteolytic lesion in the right angle of the jaw in an 82 year old lady. The tumour consisted of medium-to-large pleomorphic cells with numerous mitoses. Desmin and myogenin, indicating rhabdomyosarcoma, and EMA and cytokeratin, indicating carcinoma, were positive. The diagnosis was, therefore, between a sarcomatoid carcinoma with rhabdomyoblastic differentiation or a pleomorphic rhabdomyosarcoma with aberrant cytokeratin immunophenotype. Electron microscopy on multiple blocks revealed no

epithelial features, but cytoplasm contained many sarcomeric filaments with primitive Z-disks, thereby confirming rhabdomyosarcoma.

Case 6 This was a large gluteal myxoid malignant tumour in a 57 year-old man, in which the tumour formed clusters, strands and loops in a pattern reminiscent of extraskeletal myxoid chondrosarcoma. However, tumour cells were larger and more pleomorphic. Tumour cells contained brightly eosinophilic granules which the pathologist speculated might be large rER cisternae containing the microtubular structures known to be typical of extraskeletal myxoid chondrosarcoma. Critical electrolyte concentration staining indicated chondroid matrix, and cytokeratin was equivocal. In the context of this diagnostic uncertainty a wide panel of immuno-markers was tested and synaptophysin and chromogranin were positive. The diagnosis was difficult: a variant of extraskeletal myxoid chondrosarcoma and a tumour showing cartilaginous differentiation (for example, a mucoid carcinoma of sweat-gland origin) were considered. Electron microscopy revealed abundant mitochondria, neuroendocrine granules, and a finely textured "chondroid" matrix, all consistent with the very rare neuroendocrine myxoid chondrosarcoma.

Electron microscopy can also subtype tumours which are convincingly diagnosed as a sarcoma or a mesenchymal tumour but where the cellular differentiation is uncertain on the basis of light microscopy.

Case 7 was of a left upper arm lesion in a 17 year-old girl showing round-cell morphology and infiltrating muscle. Immunostaining was positive for neurofilament, cytokeratin, NSE, PGP9.5, and MIC2. Synaptophysin and chromogranin were negative. These markers suggested PNET but there were also features of proximal epithelioid sarcoma. Electron microscopy revealed unambiguous neuroendocrine granules, indicating PNET.

Case 8 A case of proximal epithelioid sarcoma in a 31 year-old man. The specimen was a lymph node metastasis: an earlier mass of identical morphology had been excised from the thigh. The tumour consisted of epithelioid and rhabdoid cells, which were positive for vimentin and cytokeratin, CD34 and EMA. Electron microscopy revealed prominent rER and intermediate filaments and no epithelial ultrastructure, consistent with epithelioid sarcoma.

Sometimes EM produces unexpected findings, on the basis of light microscopy histology and immunostaining.

Case 9 was a sarcoma of the soft tissue in the inguinal region in a 65 year-old man. Histology indicated a pleomorphic poorly differentiated sarcoma. There was good immunostaining evidence for both smooth- and striated-muscle cell differentiation. Electron microscopy found no evidence of striated muscle differentiation and only modest smooth-muscle myofilaments. The cytoplasm was dominated by abundant rough endoplasmic reticulum and some cells had a macrophage morphology, with a overall indication of "fibro-histiocytic" differentiation.

Case 10 This was a tumour attached to the sigmoid colon of a young woman, aged 18. It had spindle-cell morphology with smooth-muscle actin and desmin but also c-kit staining. It lacked the classical morphology of GIST, having also some large almost ganglion-like cells. GIST and inflammatory myofibroblastic tumour were considered in the differential

diagnosis but the weight of evidence, including electron microscopy (which indicated myofibroblastic differentiation), suggested an inflammatory myofibroblastic tumour.

SUMMARY

Electron microscopy continues to be useful in confirming, refining and correcting light microscope diagnoses of problematical soft-tissue tumours. Tumours continue to be encountered by pathologists where (a) the distinction between sarcoma or mesenchymal tumour, on the one hand, and malignant melanoma or carcinoma, on the other, is rendered more confidently, and (b) a mesenchymal tumour is subclassfied more precisely by ultrastructural means. In particular, electron microscopy is often called on to clarify a problem arising from conflicting or uncertain immunohistochemical findings. The potential role of electron microscopy in research and academic pathology is undiminished.

REFERENCES - GENERAL

1. Kandel R et al. Value of electron microscopy and immunohistochemistry in the diagnosis of soft tissue tumors. Ultrastruct Pathol 1998, 22: 141-146
2. Mierau G. Electron microscopy for tumour diagnosis: not redundant – resurgent! (Part of "Expert Opinion" feature: Electron microscopy for tumour diagnosis: is it redundant?). Histopathology 1999, 35: 99-102
3. Eyden B. Electron microscopy in tumour diagnosis: continuing to complement other diagnostic techniques (part of "Expert Opinion": Electron microscopy for tumour diagnosis: is it redundant?). Histopathology 1999, 35: 102-108
4. Lloreta-Trull J et al. Electron microscopy in pathology articles: a retrospective appraisal. Ultrastruct Pathol 2000, 24: 105-108
5. Tucker JA. The continuing value of electron microscopy in surgical pathology. Ultrastruct Pathol 2000, 24: 383-389
6. Eyden B. Electron Microscopy in the Diagnosis of Tumours. Current Diagn Pathol (in press) 2002
7. Sawada M et al. Establishment and characterization of human uterine leiomyosarcoma heterotransplanted into nude mice. Int J Cancer 1992, 52: 124-129
8. Pantuck AJ et al. Diagnosis and management of malignant perirenal schwannoma. Amer Surg 1996, 62: 1024-1027

SELECTED REFERENCES ON SPECIFIC TUMOUR-TYPES

Schwannoma/Malignant peripheral nerve sheath tumour:

1. Dickersin GR. The electron microscopic spectrum of nerve sheath tumors. Ultrastruct Pathol 1987, 11: 103-146

Atypical fibroxanthoma:

1. Alguacil-Garcia A et al. Atypical fibroxanthoma of the skin. An ultrastructural study of two cases. Cancer 1977, 40: 1471-1480
2. Barr RJ et al. Ultrastructure of atypical fibroxanthoma. Cancer 1977, 40: 736-743

PNET:

1. Cavazzana AO et al. Peripheral neuroepithelioma: a light microscopic, immunocytochemical, and ultrastructural study. Mod Pathol 1992, 5: 71-78

2. Lombart-Bosch A et al. Peripheral neuroectodermal sarcoma of soft-tissue (peripheral neuroepithelioma): a pathologic study of 10 cas with differential diagnosis regarding other small, round-cell sarcomas. Hum Pathol 1989, 20: 273-280

Sclerosing epithelioid fibrosarcoma:

1. Meis-Kindblom JM et al. Sclerosing epithelioid fibrosarcoma. A variant of fibrosarcoma simulating carcinoma. Amer J Surg Pathol 1995; 19: 979-993
2. Reid R et al. Sclerosing epithelioid fibrosarcoma. Histopathology 1996; 28: 451-455
3. Eyden BP et al. Sclerosing epithelioid fibrosarcoma: a study of five cases emphasizing diagnostic criteria. Histopathology 1998; 33: 354-360
4. Antonescu CR et al. Sclerosing epithelioid fibrosarcoma. A study of 16 cases and confirmation of a clinicopathologically distinct tumor. Amer J Surg Pathol 2001; 25: 699-709

Rhabdomyosarcoma:

1. Erlandson RA. The ultrastructural distinction between rhabdomyosarcoma and other undifferentiated "sarcomas". Ultrastruct Pathol 1987, 11: 83-101

Neuroendocrine myxoid chondrosarcoma:

1. Harris M et al. Extraskeletal myxoid chondrosarcoma with neuroendocrine differentiation: a pathological, cytogenetic and molecular study of a case with a novel translocation (9;17)(q22; q11.2). Amer J Surg Pathol 2000; 24: 1020-1026
2. Goh et al. Extraskeletal myxoid chondrosarcoma: a light microscopic, immunohistochemical, ultrastructural and immuno-ultrastructural study indicating neuroendocrine differentiation. Histopathology 2001 39: 514-524

Proximal epithelioid sarcoma:

1. Guillou L et al. "Proximal-type" epithelioid sarcoma, a distinctive aggressive neoplasm showing rhabdoid features. Am J Surg Pathol 21: 130-146, 1997

Malignant fibrous histiocytoma:

1. Lagacé R. The ultrastructural spectrum of malignant fibrous histiocytoma. Ultrastruct Pathol 1987, 11: 153-159
2. Jabi M et al. Ultrastructural heterogeneity in malignant fibrous histiocytoma of soft tissue. Ultrastruct Pathol 1987, 11: 583-592

Gastrointestinal stromal tumour:

1. Erlandson RA. Subclassification of gastrointestinal stromal tumors based on evaluation by elctron microscopy and immunohistochemistry. Ultrastruct Pathol 1996, 20: 373-393

Inflammatory myofibroblastic tumour:

1. Gilks CB et al. Inflammatory pseudotumor of the uterus. Int J Gynecol Pathol 6: 275-286, 1987
2. Lundgren L et al. Pseudomalignant spindle cell proliferations of the urinary bladder. Hum Pathol 25: 181-191, 1994
3. Corsi A et al. Recurrent inflammatory myofibroblastic tumor of the glottis mimicking malignancy. Am J Otolaryngol 18: 121-126, 1997

QUESTIONS

1. In a tumour containing structures suggestive of vascular spaces and therefore resembling angiosarcoma but staining strongly with anti-cytokeratin antibodies, which two cell organelles would identify a pseudo-angiosarcomatous squamous cell carcinoma?
   Name them: ....., ...
2. Name one cell organelle and one immuno-marker which together would unambiguously identify the neuroendocrine subtype of myxoid chondrosarcoma.
   Cell organelle: .....;
   Immuno-marker: .....

1. In un tumore di architettura pseudovascolare, positivo per citokeratine, quali sono i due reperti oganellari cellulari che lo qualificherebbero come carcinoma squamoso pseudoangiosarcomatoso?.
   Nominare i due reperti organellari: .....;
   .....
2. Nomina un organulo cellulare e un marker immunoistochimico che inequivocabilmente qualificherebbero un condrosarcoma mixoide della varietà neuroendocrina.
   Organulo cellulare: .....;
   Marker immunoistochimico: .....

SURGICAL PATHOLOGY -SESSION I. UROGENITAL TRACT I

CASE PRESENTATIONS (4 cases)

Case 1. Pseudocarcinomatous epithelial hyperplasia with mural pseudoinvasion in acute and chronic non-tuberculous salpingitis -chlamydia infection

(D. Ben-Dor)

Case 2. Malignant mesothelioma of the tunica vaginalis testis

(M. Bisceglia)

Case 3. Malignant Sertoli cell tumor of the testis

(S. Suster)

Case 4. Primary endometrioid FATWO-like carcinoma of the uterine salpynx

(M. Fukunaga)

CASE 1. 1729-01

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CLINICAL HISTORY

The patient was a 22 year old female in general good health. In the past she had two pregnancies and one delivery. At the time of presentation she was using an IUD for contraception. Otherwise her gynecologic history was not noteworthy. Menses were generally regular. Two months prior to investigation, ultrasound examination performed due to a complaint of vaginal bleeding revealed a right ovarian cyst about 8 cm. in diameter with papillations. No other abnormal findings were indicated. There is no mention in the patient file of possible febrile illness with pelvic or abdominal pain. Subsequent laparoscopic examination revealed a right adnexal conglomerate containing a thickened fallopian tube adherent to the sigmoid colon and the posterior wall of the uterus. The ovary was enlarged and contained a few cysts. The left fallopian tube was also enlarged (but to a lesser degree than on the right) with numerous adherences to the uterus, and the ovary was normal. After releasing the adherences the right fallopian tube (without the ovary) was excised and sent to pathology with the description, "right tubal mass".

PATHOLOGY

The specimen consisted of a fallopian tube segment which measured 8x2 cm., showing mostly sausage like diffuse enlargement. One end was gaping and the other narrower; no fimbriae were noted. On sectioning the interior showed friable soft contents. There was no macroscopic mass formation.

Histology shows on low power the inner epithelial layer to be thickened and hypercellular. On close examination, there is an intense acute and chronic inflammatory infiltrate with many plasma cells. What is particularly noteworthy are the changes in the epithelial structures themselves. In many places the glands are fused forming cribriform sheets. Here and there one might think that the glandular lumens are effaced with the cells forming solid foci. The nuclei are enlarged, pale, and crowded, here and there also giving the impression of being stratified. What was particularly disturbing was the fact that though on low power the cellular inner layer looks well demarcated from the muscularis, on high power one can detect glands entrapped between smooth muscle fibers.

I must admit that I was extremely troubled by these findings. Though at least theoretically aware of the fact that cases of salpingitis may show marked and confusing pseudoneoplastic epithelial changes, not having seen personally cases like this I didn't realize that these may be extreme and include features commonly associated with malignancy in other contexts, such as cribriforming, as well as (pseudo)infiltration of muscle. Fortunately, before any serious damage could be done, a colleague pointed out to me the relevant description in the current AFIP fascicle (1) which illustrates all these findings as non-neoplastic in the context of severe inflammatory disease, either granulomatous or non-granulomatous. This phenomenon in the context of non-granulomatous disease is also discussed in an article published by Cheung et al (2). The cases included in this paper involve mostly younger women (17-40 yrs.). Additional features listed by them and not illustrated in this case include desmoplastic response, presence groups of epithelial cells in vascular lumens, psammoma body formation, and mesothelial hyperplasia, adding to the potential confusion. In this context, malignancy should NOT be diagnosed in the absence of the following features: a solid mass

macroscopically, increased mitotic activity with atypical figures, and severe atypia. Reviewing the slides critically once again in a cooler and more informed frame of mind, none of these features are found in this case. Furthermore, the age of this patient is not characteristic of tubal carcinomas (usually seen in older women). Further, one can detect here and there a few glands at the periphery of the intense proliferation but in continuity with it which look benign.

DIAGNOSIS

Pseudocarcinomatous epithelial hyperplasia with mural pseudoinvasion in acute and chronic non-tuberculous salpingitis.

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Since seeing is believing, I thought it worthwhile to present this case so that those who don't have first hand acquaintance with this condition will keep it in mind should they confront this situation in their own practice.

DISCUSSION

Pseudomalignant changes have been known to be present in the context of tuberculous salpingitis for a long time. Non-granulomatous salpingitis is commonly associated with gonorrheal or chlamydial infection. These infections spread upward from the lower genital tract. The fallopian tube changes are at first purulent followed by the appearance of lymphocytes and many plasma cells. Chlamydia may be associated with cytoplasmic inclusions in cytologic preparations but to the best of my knowledge there is no way to infer the presence of this organism or identify it on routinely stained material. Inflammation usually leads to fusion of the plicae and epithelial herniation beyond the mucosa into the muscularis; in most instances this is not misinterpreted. The end result might be hydrosalpinx with dilated glandular spaces formed by these processes. When the process is quiescent there would be no place to suspect malignancy. However in the acute and subacute phase, these changes when taking on a particularly florid aspect as in this case might cause great confusion.

Upon confirmation of the diagnosis of a non-neoplastic condition the patient was released from the hospital and since then has not returned with any complaints referable to the condition for which she was treated here. I am not aware of any further workup performed inside or outside the hospital. However in the course of preparing this case for this presentation I sent a block to Dr Allen Gown of Phenopath Laboratories, Seattle WA USA, a member of the AMR club, to see if the presence of chlamydia could be confirmed immunohistochemically. He was kind enough to perform the test using a non-commercial antibody, CF2 (developed by Dr Dorothy Patton, a chlamydia investigator at the University of Washington). Chlamydia antigen was indeed identified focally in epithelial cells at the luminal surface, a pattern observed by Dr Gown in animal tissues studied in investigations carried out with Dr Patton (personal communication).

The issue of etiology was not specifically investigated in the paper by Cheung et al and I don't know of anyone who has specifically chlamydia antigen in cases like the one I am discussing.

In passing and order to complete the discussion non-neoplastic tubal epithelial proliferations may be seen as a result of estrogenic stimulation from various causes, and in

the presence of neoplasia elsewhere in the genital tract not directly involving the tubes, as a "field effect".

FINAL DIAGNOSIS

Pseudoneoplastic epithelial proliferation in florid acute and chronic salpingitis positive for chlamydia infection.

REFERENCES

1. Tumors of the ovary, maldeveloped gonads, Fallopian tube, and broad ligament Atlas of Tumor Patholohy, Third Series (No. 20), Armed Forces Institute of Pathology, 1998, p. 485-486
2. Cheung A.N.Y., Young R. H., and Scully R. E., Pseudocarcinomatous hyperplasia of the Fallopian tube associated with salpingitis. Am J Surg Pathol 18: 1125-1130, 1994

QUESTIONS

1. The presence of cribriforming and muscular penetration of atypical glands in Fallopian tube lesions is always diagnostic for malignancy, regardless of the setting and age of the patient. **True or False.** _____.
2. Pseudoneoplastic epithelial proliferations in the Fallopian tube are not always tuberculous in etiology. **True or False.** _____.

1. La presenza di ghiandole atipiche intramurali nelle lesioni della tuba di Falloppio rappresenta sempre una espressione di malignità, a prescindere dal contesto clinico del paziente. **Vero o Falso.** _____.
2. I processi proliferativi epiteliali atipici (pseudoneoplastici) non sono sempre secondari a una etiologia tubercolare. **Vero o Falso.** _____.

CASE 2. 17249-0

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CLINICAL HISTORY

A 74-year old male patient was admitted complaining of testicular pain on the right side. The past medical history recorded a previous surgical intervention of a homolateral marsupialization for hydrocele, which was performed less than 2 years before. At physical examination an intrascrotal paratesticular mass was noted. An ultrasound scan revealed a paratesticular solid-cystic mass of 4-5 cm in size with testis involvement. The patient underwent a radical right inguinal orchiectomy.

PATHOLOGY

GROSS FINDINGS

The visceral tunica vaginalis was partly coated by an apparently papillary and exophytic tumor. On sectioning the tumor was seen infiltrating the adjacent testicular parenchyma, the epididymis, and the spermatic cord.

MICROSCOPIC FINDINGS

The histologic examination revealed an epithelioid tumor with tubulo-papillary pattern. On the vaginal surface the tumor showed a predominant papillary pattern while in the invasive component it exhibited a variety of less-differentiated patterns with tubular, tubulo-papillary and even foci of solid components. Spindle or sarcomatous pattern was not a feature. Some well differentiated papillary excrescences were also distributed aside the main tumor mass on the external surface. Cytologic atypia was evident. Mitotic activity was very low. Very rare psammoma bodies were also seen. PAS stain failed to reveal any cytoplasmic mucin production. Immunohistochemically the tumor was positive for keratins (cocktail), EMA, and calretinin, and negative for CEA and LeuM1.

ELECTRON MICROSCOPICAL FINDINGS

Ultrastructurally the main feature was the presence of numerous slender microvilli of the mesotheliomatous type on cell surface. Numerous desmosomes with some of them particularly long (>700 nm), abundance of intracytoplasmic filaments and scarcity of cytoplasmic organelles were noted in tumor cells.

DIAGNOSIS

Malignant epithelial mesothelioma of the tunica vaginalis testis –paratesticular malignant mesothelioma.

DISCUSSION

Paratesticular malignant mesotheliomas -almost synonymic with malignant mesothelioma of tunica vaginalis testis- are rare tumors. Since the first description by Barbera and Rubino (1) up to 1995 sixty-four such cases were found in the literature by Jones et al (2) who reported on their own the most consistent series of 11 cases so far appeared. In 1998 based on a computerized search of the literature Plas et al (3) established at 74 the number of the total cases on record for the previous 30 years (1966 to 1997) to which they added an additional personal case and to which 9 more cases should be added which were included by Jones et al and ignored by Plas et al. Actually in our most recent updating review of the literature (1985-April 2002) we were able to find 32 more cases since 1997 (4-25) and to add as many as 25 further cases (26-30, 32-49) relevant to the interval time (1985-1997) already encompassed in the previous above main searches (2,3), so making a grand total of at least 141 cases ever described up to April 2002. Personal unpblished

experience with mesotheliomas of this area include: 2 cases of malignant mesothelioma of tunica vaginalis, a case of malignant mesothelioma of peritoneum in an inguinal hernial sac, and a case of benign multicystic mesothelioma of spermatic cord. The case here presented is one of the two above mentioned malignant tunical examples.

Paratesticular malignant mesotheliomas have a wide age range distribution (7-87 years), mainly occurring in patients in their VI-VIII decades of life, with 1/5 of patients in the first three decades. Occasionally even children can be affected (in ref. 3: cf. 42; 32). The most frequent presenting symptom is represented by scrotal enlargement with hydrocele in over 55% of cases and a paratesticular mass in over 30%. A history of direct or familial asbestos exposure is detectable in

34 % of cases (in ref 2:cf 21,53; 3,22,31,40,44). In exceptional cases a bilateral involvement has been described (in ref. 3: cf. ref 35) and even a simultaneous involvement of serosal membranes of different cavities (17,49) have been observed. The preoperative clinical diagnosis has never been established or firmly suspected (2,3). Only four-five cases (in ref 3: cf. 14; 7, 24, 48) had their preoperative cytology fluid/FNA-based diagnosis. The diagnosis is often suspected intraoperatively and always needs a histological confirmation or recognition. Paratesticular mesotheliomas usually arise from the mesothelial layer of the parietal or visceral surface of tunica vaginalis testis (50-51), although included in the same rubric of tumors are very rare examples arisen from the mesothelium of a hernial sac (13) or from the mesothelium covering the spermatic cord, the latter locations accounting for less than 10% of cases (present review). Microscopically the tumor is most often of the pure epithelial type or mixed, with only exceptional cases on record of the sarcomatous spindle cell type (in ref. 2: 17). The architectural pattern is usually papillary or tubulo-papillary and is identical to the corresponding histotypes of the abdominal and thoracic counterparts. Rare examples of well-differentiated papillary mesotheliomas resembling the benign variant of the female peritoneum have also been described in young patients (19,35,36). The differential diagnosis of paratesticular mesothelioma mainly include some mullerian epithelial neoplasms (52-55) -possibly arising from mullerian remnants or from peritoneal mullerian metaplasia-, and adenocarcinoma of rete testis. However a miscellaneous list of primaries of different histogenesis (56-58) also should be taken into account in differential, such as papillary cystadenoma of the epididymis, adenocarcinoma of the epididymis, adenomatoid tumor (benign nonpapillary mesothelioma), multicystic mesothelioma of the spermatic cord (59), reactive mesothelial hyperplasia, so-called benign papillary mesothelioma (60), and metastastic cancer (lung, prostate). In very rare cases (poorly differentiated mesotheliomas) with invasion of testicular parenchyma, also Leydig cell tumor might enter the differential diagnosis (parenthetically Leydig cell tumor is also calretinin positive which is the most reliable marker in the diagnosis of mesothelioma [61]).

Histochemically: PAS stain and mucicarmine stain are negative. Alcian blu shows hyaluronic acid positivity removed by hyaluronidase. Immunohistochemically –like the homologues of the main body cavities- paratesticular mesotheliomas are positive for cytokeratin (cocktails), cytokeratin 7, cytokeratin 5/6, EMA (membrane pattern), calretinin. Negative results are from immunostains for: cytokeratin 20, BerEP4, B72.3, MOC-31, HMFG-2, LeuM1, CEA, ER, PR. LeuM1 and CEA may sometimes give inappropriate staining (62), which should be removed by a pretreatment of sections with hyaluronidase (63). Vimentin may give different results form focal to diffuse positive to negative. Some mesothelial antibodies (HBME-1) are not completely reliable since they immunoreact also with a good deal of carcinomas.

Anyhow the goldstandard for the diagnosis of mesothelioma (of any site) in dubious cases remains electron microscopy which easily shows the main fine morphological feature

represented by the long sinuous microvilli on the tumor cell surface (in ref 2: cf 10,17,27,36,37,42; 4, 5,).
Paratesticular malignant mesotheliomas are aggressive neoplasm capable of widespread local involvement as well as lymphatic and hematogenous metastases which can not only supervene as a disease progression in cases with already established diagnosis, but also present as a primarily locally advanced or disseminated disease at diagnosis (3), with a final lethal course in 30% of cases after a median survival of 24 months. The first-line surgical treatment is represented by inguinal radical orchiectomy. Conservative approach should be discouraged being almost always complicated by local recurrences. Radiation therapy and chemotherapy have are not helpful in the treatment or control of this disease (8).

Follow-up : 18 months after the surgical intervention the patient is alive and free of disease. No adjuvant therapy administered.

REFERENCES

1. Barbera V, Rubino V. Papillary mesothelioma of the tunica vaginalis. Cancer 1957; 10: 182-9.
2. Jones MA, Young RH,and RE Scully. Malignat mesothelioma of the tunica vaginalis. A clinicopathologic analysis of 11 cases with review of the literature. Am J Surg Pathol 1995; 19: 815-825.
3. Plas E, Riedl CR, Pfluger H. Malignant mesothelioma of the tunica vaginalis testis. Review of the literature and assessment of prognostic parameters.Cancer 1998; 83:2437-46.
4. Agapitos E, Pavlopoulos PM, Marinos E, et al. Malignant mesothelioma of the tunica vaginalis testis: an immunohistochemical and ultrastructural study of 2 case. Br J Urol 1997; 80:345-6.
5. Berti E, Schiaffino E, Minervini MS, et al. Primary malignant mesothelioma of the tunica vaginalis testis. Imunohistochemistry and electronmicroscopy. Pathology 1997; 29:96-9.
6. Wakasugi E, Ishii T, Akiyama T, et al. A case of malignant mesothelioma associated intrascrotal mass. Nippon Hinyokika Gakkai Zasshi 1997; 88:443-6.
7. Gupta SC, Gupta AK, Misra V, et al. preoperative diagnosis of malignant mesothelioma of tunica vaginalis testis by hydrocele fluid cytology. Eur J Surg Oncol 1998; 24:153-4.
8. Gupta NP, Aggrawal AK, Sood S, et al. Malignant mesothelioma of the tunica vaginalis testis: a report of two cases and review of the literture. J Surg Oncol 1999; 70:251-4.
9. Hamm M, Rupp C, Rottger P, et al. Malignant mesothelioma of the tunica vaginalis testis. Chirur 1999; 70: 302-5.
10. Harmse JL, Evans AT, Windsor PM. Malignant mesothelioma of the tunica vaginalis: a case with an unusually indolent course following radical orchiectomy and radiotherapy. Br J Radiol 1999; 72:502-4.
11. Janssen-Hejnen ML, Damhuis RA, Klinkhamer PJ, et al. Increased but low incidence and poor survival of malignant mesothelioma in the southeastern part of the Nederlands since 1970: a population-based study. Eur J Cancer Prev 1999; 8:311-4.
12. Kanazawa S, Nagae T, Fujwara T, et al. Malignant mesothelioma of the tunica vaginalis testis: report of a case. Surg Today 1999; 29:1106-10.
13. Aggarwal M, Lakhar B, Shetty D, et al . Malignant peritoneal mesothelioma in an inguinal hernial sac: an unusual presentation. Indian J Cancer 2000; 37:91-4.

14. Coue O, Depinieux G, Lami ML, et al. A tunica vaginalis testicular tumor in a 20-year old man. Ann Pathol 2000; 20: 633-4.
15. Ferri E, Azzolini N, Sebastio N, et al. Unusual case of mesothelioma of the tunica vaginalis associated with prostatic adenocarcinoma . Minerva Urol Nefrol 2000; 52:33-5.
16. Fujisaki M, Tokuda Y, Sato S, et al. Case of mesothelioma of the tunica vaginalis testis with characteristic findings on ultrasonograhy and magnetic resonance imaging. Int J Patholl 2000; 7:427-30.
17. Poggi A, Longo F, Mansueto G, et al. A case of mesothelioma of the tunica vaginalis testis, with involvement of the pleura and peritoneum. Tumori 2000; 86:256-7.
18. Uria Gonzales-Tova J, Escalera Almendros C, et al. Malignant mesothelioma of the tunica vaginalis. Actas Urol Esp 2000; 24:757-60.
19. Xiao SY, Rizzo P, Carbone M. Benign papillary mesothelioma of the tunica vaginalis testis. Arch Pathol Lab Med 2000; 124:143-7.
20. Gurdal M, Erol A. Malignant mesothelioma of tunica vaginalis testis associated with long-lasting hydrocele: could hydrocele be an etiological factor. Int Urol Nephrol 2001; 32:687-9.
21. Murai Y. Malignant mesothelioma In Japan: analysis of registered autopsy cases. Arch Environ Health 2001; 56: 84-8.
22. Schneider J, Woitowitz HJ. Asbestos-induced malignant mesothelioma of the tunica vaginalis testis. Zentrabl Chir 2001; 126:229-32.
23. Wolanske K, Nino-Murcia M. Malignant mesothelioma of the tunica vaginalis testis: atypical sonographic appearance. J Ultrasound Med 2001; 20:69-72.
24. Bruno C, Minniti S, Procacci C. Diagnosis of malignant mesothelioma of the tunica vaginalis testis by ultrasound-guided fine needle aspiration. J Clin Ultrasound 2002; 30:181-3.
25. Iczkowski KA, Katz J, Zander DS, et al. Malignant mesothelioma of the tunica vaginalis testis: a fatal case with liver metastasis. J Urol 2002; 167:645-6.
26. Aquino NM, Vasquez R, Matari H. Ultrasound demonstration of a benign mesothelioma of tunica vaginalis testis. J Clin Utrsound 1986;14:310-1.
27. Rey Lopez A, Redondo Martinez E, Burgos Lazaro F, et al. Mesothelioma of the tunica vaginalis testis. Arch Esp Urol 1989; 42:157-60.
28. Gotoh S, Mine M, Ishizaka K, et al. Malignant mesothelioma of the tunica vaginalis testis. Hinyokika Kiyo 1989; 35. 1973-5.
29. Malamizzi-Puchner A, Dimitriadis D, Bartsokas, et al. Proteus syndrome: course of a severe case. Am J Med Genet 1990; 35:283-5.
30. Rodriguez Andres JA, Garmendia Larrea JC, Hernandez Lecuona I, et al. Diffuse malignant mesothelioma of the tunica vaginalis testis. Report of a new case. Arch Esp Urol 1990; 43.897-9.
31. Mirabella F. Malignant mesothelioma of the tunica vaginalis testis. Here again asbestos? Minerva Med 1991; 82: 765-70.
32. Pfister M, Saez D, Celeste F. Sonographic appearance of malignant mesothelioma of the tunica vaginalis testis in a child. J Clin Ultrasound 1992; 20:129-31.
33. Fields JM, Russel SA, Andrew SM. Case report: ultrasound appearances of a malignant mesothelioma of the tunica vaginalis testis. Clin Radiol 1992; 46:128-130.
34. Bierman CV, Gasser TC, Rutishauser G. Malignant mesothelioma of the tunica vaginalis testis. Helv Chir Acta 1992; 59:501-2.
35. Chetty R. Well differentiated (benign) papillary mesothelioma of the tunica vaginalis. J Clin Pathol 1992; 45:1029-30.
36. Fujii Y, Masuda M, Hirokawa M, et al. A case of benign mesothelioma of the tunica vaginalis testis. Hinyokika Kiyo 1993; 39:89-92.

37. Saw KC, Barker TH, Khalil KH,et al. Biphasic malignant mesothelioma of the tunica vaginalis testis. Br J Urol 1994, 74:381-2.
38. Reynard JM, Hasan N, Baithun SI, et al. Malignant mesothelioma of the tunica vaginalis testis. Br J Urol 1994; 74:389-90.
39. Borrell Planca A, Alapont Perez M, Compan Quilis A, et al.. Malignant mesothelioma of the tunica vaginalis testis. Actas Urol Esp 1994; 18:899-902.
40. Watanabe M, Kimura N, Kato, et al. An autopsy case of malignant mesothelioma associated with asbestosis. Pathol Int 1994; 44: 785-92.
41. Bierman CW, Moch H, Gasser TC, et al. Primary detection of malignant mesothelioma of the tunica vaginalis testis using the BMA-120 monoclonal antibody. Helv Chir Acta 1994; 60: 1127-9.
42. 42. Joseph AK, Tzchen JA, Sekula Sa, et al. Putpuric nodules along a surgical scar. A metastatic Malignant mesothelioma of the tunica vaginalis testis. Arch Dermatol 1995; 131:484-8
43. Lopez JI, Angulo JC, Ibanez T. Combined therapy in a case of malignant mesothelioma of the tunica vaginalis testis. Scand J Urol Nefrol 1995; 29:361-4.
44. Huncharek M, Klassen M, Christiani D. Mesothelioma of the tunica vaginalis testis with possible asbestos exposure. Br J Urol 1995; 75:679-80.
45. Umekauwa T, Kurita T. Treatment of mesothelioma of the tunica vaginalis testis. Urol Int 1995; 55:215-7.
46. Magoha GA. Testicular cancers in Nigerians. East Afr Med J 1995; 72:554-6.
47. Pellice Vilalta C, Cosme Jimenez M, Casalots Serramia J. Neoplasms of the tunica of the testis. Report of 5 cases (3 mesothelial tumors and two fibrous pseudotumors). Arch Esp Urol 1996; 49:12-6.
48. Ahmed M, Chari R, Mufi GR, et al. Malignant mesothelioma of the tunica vaginalis testis diagnosed by aspiration cytology –a case report with review of literature. Int Urol Nephrol 1996; 28: 793-6.
49. Ascoli V, Facciolo F, Rahimi S, et al. Concomitant malignant mesothelioma of the pleura, peritoneum,and tunica vaginalis testis. Diagn Cytopathol 1996; 14:243-8.
50. Roggli VL, Kolbeck J, Sanfilippo F, et al. Pathology of human mesothelioma. Etiologic and diagnostic considerations. Pathol Annu 1987 (part II): 91-126.
51. Walker AN and Mills SE. Surgical Pathology of the tunica vaginalis testis and embryologically Related mesothelium. Path Annu 1988 (part 2): 125-150.
52. Young RH, Scully RE. Testicular and paratesticular tumors and tumor-like lesions of ovarian common epithelial and Mullerian types. Am J Clin Pathol 1986; 86:146-52.
53. Becerra P, Isaac MA, Márquez B, et al. Papillary serous carcinoma of the tunica vaginalis testis. Pathos Res Pract 2000; 196: 781-782.
54. McClure RF, Keeney GL, Sebo TJ, et al. Serous borderline tumor of the paratestis. A report of seven cases. Am J Surg Pathol 2001; 25: 373-378.
55. Caccamo D, Socias M, Truchet C. Malignant Brenner tumor of the testis and epidydimis. Arch Pathos Lab Med 1991; 115:524-527.
56. Boswick DG, Eble JN. Urologic Surgical Pathology. Mosby, St Louis. 1997:658-663.
57. Ulbright TM, Amin MB, and Young RH. Tumors of the adnexa, spermatic cord, and scrotum. Atlas of Tumor Pathology. 3rd Series. Fascicle 25. Armed Forces Institute of Pathology. Washington D.C: 1999: 235-290.
58. Srigley JR and Hartwick WJ. Tumors and cysts of the paratesticular region. Path Annu 1999 (part 2): 51-100.
59. Tobioka H, Manabe K, Matsuoka S, et al. Multicystic mesothelioma of the spermatic cord. Histopathology 1995; 27: 479-481.
60. Chahla Y. Benign genital mesothelioma. Two case report. Eur Urol 1985; 11:285-287N

61. Doglioni C, De Tos P, Laurino L, et al. Calretinin: a novel immunocytochemical marker for mesothelioma. Am J Surg Pathol 1996; 20:1037-1046.
62. Stirling JW, Henderson DW, Spagnolo DV, et al. Unusual granular reactivity for carcinoembryonic antigen in malignant mesothelioma. Hum Pathol 1990; 21:678-679.
63. Robb JA. Mesothelioma versus adenocarcinoma: false-positive CEA and Leu-M1 staining due to hyaluronic acyd. Hum Pathol 1989; 20: 400 (letter).

QUESTIONS

1. A. Which is the best current imunocytochemical marker in the diagnosis of mesothelioma?
   Indicate the name: .....
   B. Which is the most diagnostically characteristic ultrastructural finding in mesothelioma?
   Indicate the finding: .....
2. Which are the malignant tumors mainly entering the differential diagnosis versus paratesticular mesothelioma?
   Indicate the main two: .....,
   .....

1. A. Quale è il miglior marker attualmente impiegato in imunocitochimica per la diagnosi di mesotelioma?
   Specificare il nome: .....
   B. Quale è il reperto ultrastrutturale più caratteristico e significativo sul piano diagnostico, che si osserva nel mesotelioma?
   Indicare il reperto: .....
2. Quali sono i tumori maligni che entrano principalmente in diagnosi differenziale con il mesotelioma paratesticolare?
   Indicare i due principali: .....;
   .....

CASE 3. 8455

Contributor: Saul Suster, M.D., Director of Pathology, The Ohio State University, Columbus, Ohio, USA.

CLINICAL HISTORY

An 81 year old man with no significant past history was seen for swelling of his left testis. At surgery, a tan fleshy tumor measuring 3.7 x 2.9 x 2 cm was found within the testicular parenchyma. An orchiectomy was done.

PATHOLOGIC FINDINGS

Histologic examination revealed a heterogenous proliferation of cellular elements with a variegated appearance. Some areas of the tumor were composed of tubular elements containing cells with abundant pale, clear cell or lipid-rich cytoplasm typical of Sertoli cell tumors of the testis. Other areas, however, showed a denser, round to spindle cell proliferation displaying clear transitions with the clear cell tubular elements. The round cell areas contained nuclei with similar features to those of the tubular areas, but the cytoplasm was scantier and showed a more eosinophilic tinctorial appearance. The tumor cells formed cords and packets that were separated by strands of fibroconnective tissue. In some areas, the cells appeared to become discohesive resulting in a vessel-like pattern reminiscent of vascular neoplasia. The solid, spindle cell component (seminar slide) was characterized by sheets and cords of tightly packed oval to spindle cells separated by strands of collagenous tissue. The cells displayed large, vesicular nuclei with occasional eosinophilic nucleoli and scattered mitotic figures (averaging 5-10 per 10 HPF). In some areas, the spindle cell proliferation merged with a focal glandular component displaying a retiform pattern, with the glands being lined by cells displaying similar features to those of the invasive component. Areas of tumor necrosis were evident in several fields, and the tumor cells were seen to penetrate the tunica vaginalis and the rete testis. Vascular space invasion was also evident.

SPECIAL STUDIES

Immunohistochemical studies showed positivity of the tumor cells for cytokeratin AE1/AE3 and vimentin, and negative staining for alpha-fetoprotein, placental-like alkaline phosphatase, inhibin, and S-100 protein. Tissue was not available for electron microscopy.

DIAGNOSIS

Malignant Sertoli cell tumor of testis.

DISCUSSION

Sex-cord stromal tumors of the testis are rare and account for less than 4% of all testicular neoplasms.[1] These tumors are most common in prepubertal males.[2] The cells usually resemble normal Leydig cells, Sertoli cells, or nonspecific stromal cells of the testis. Sex-cord stromal tumors of the testis are generally classified into Sertoli-stromal cell tumors, granulosa-stromal cell tumors, fibroma-thecoma, mixed and unclassified.[1] Sertoli-stromal cell tumors are further subdivided into Sertoli cell tumor, Sertoli-Leydig tumor, and Leydig cell tumor.

Sertoli cell tumors show a tremendous variegation in their morphologic growth patterns and histologic appearances, and have been traditionally difficult to subclassify. For this

reason, the majority (with the exception of the large cell calcifying and sclerosing variants) are categorized as "Sertoli cell tumor, NOS" (not otherwise specified).[1] Clinically the tumors present as a painless testicular mass without any known predisposing conditions, although cases of Sertoli cell tumor in association with endocrinologic abnormalities have been described in the literature.[3] Grossly the tumors are typically well-circumscribed, yellow, or tan-white homogeneous rubbery masses with an average diameter of 3.5 cm. Foci of hemorrhage can be present but necrosis is extremely rare.

Histologically, the tumors usually display some degree of tubular differentiation, although diffuse and nodular growth patterns tend to predominate. The tubules may be round with central lumens, or elongated and devoid of a lumen. Rarely they may show a retiform pattern of growth. The tubular structures are lined by cells with abundant eosinophilic cytoplasm, but the cytoplasm may be pale due to lipid accumulation. The stroma of the tumors usually contains bands of abundant, often hyalinized connective tissue. When extensive sclerosis is present, the tumors are classified as "sclerosing Sertoli-cell tumor".[4] Another unusual variant is the so-called "Large cell calcifying Sertoli cell tumor".[5,6] Such cases are characterized by prominent areas of calcification that may take the form of psammoma-like bodies or as large, confluent, laminated plaques or nodules with calcium deposition. The latter are often associated with the Peutz-Jeghers syndrome and other endocrinological abnormalities.

In the majority of cases, the tumor cells in Sertoli cell tumors are quite uniform and bland-appearing, without prominent nucleoli, pleomorphism or mitotic activity. In rare cases, nuclear pleomorphism, hyperchromasia and mitotic activity have been observed. The latter features have been found to correlate with clinical behavior. The issue of malignancy in Sertoli cell tumors has been, however, controversial for quite some time. In a study of a large series of Sertoli cell tumors, 7/60 cases (12%) showed malignant behavior.[7] The features that correlated best with malignant behavior included: tumor diameter >5 cm., necrosis, moderate to severe nuclear atypia, vascular invasion, and mitotic rate of >5 mitoses per 10 HPF. Malignant tumors presented with metastases to regional and retroperitoneal lymph nodes, lung and bone.

DIFFERENTIAL DIAGNOSIS:

The differential diagnosis of Sertoli cell tumors includes Sertoli cell nodules (Pick's adenoma), juvenile granulosa cell tumor, and mixed sex-cord stromal tumors. Sertoli cell nodules are usually microscopic findings and are sharply circumscribed from the surrounding parenchyma. Only rarely will they attain a sufficient size to be visible macroscopically. Juvenile granulosa cell tumors have more immature-looking nuclei with conspicuous mitotic activity, and are more often encountered within the first 2 months of life. Some Sertoli cell tumors may have areas that resemble Leydig cell tumors due to a diffuse growth of cells with abundant eosinophilic cytoplasm, but are easily recognized due to their bright yellow cut surface and lack on histological examination of evidence of tubular differentiation.

The malignant variant of Sertoli cell tumor may pose more difficulties for differential diagnosis. In a recent publication, Henley et al.[8] report 13 examples of malignant Sertoli cell tumors of the testis that displayed features that were easily confused for a seminoma. The tumors often displayed a sheet-like or nested growth pattern, with dense lymphoid infiltrates, and cells containing conspicuous clear cytoplasm and prominent nucleoli. Helpful features for the differential diagnosis were the age distribution of the patients

(much younger for seminoma than malignant Sertoli cell tumor), the lack of nuclear atypia and prominent nucleoli in a subset of the neoplastic cells, lower mitotic rate, and the absence of intratubular germ cell neoplasia. Immunohistochemical stains were also felt to be of value in differential diagnosis. Stains for inhibin-α have been reported as positive in 30-90% of Sertoli cell tumors,[9,10] whereas seminomas and other germ cell tumors are negative for this marker. Likewise, PLAP positivity has been observed in the majority of cases of seminoma, while it is not expressed in Sertoli cell tumors. In the study by Henley et al,[8] however, 3/12 cases were negative for inhibin.

Other tumors that may be considered in the differential diagnosis besides classical seminoma include the spermatocytic seminoma, yolk sac tumor with solid or spindle cell growth pattern,[11] and metastases of tumors with clear cell features such as renal cell carcinoma and malignant melanoma. Adequate sampling of the lesion, a detailed clinical history, and the identification of the more characteristic tubular areas, such as in our case, should be of help for ruling out these alternative diagnoses.

REFERENCES

1. Ulbright TM, Amin MB, Young RH. Tumors of the testis, adnexa, spermatic cord, and scrotum. Atlas of Tumor Pathology, 3rd. series, fascicle 25. Washington, D.C., Armed Forces Institute of Pathology, 1999.
2. Kaplan GW, Cromie WJ, Kelalis PP et al. Gonadal stromal tumors: a report of the prepuberal testicular tumor registry. J Urol 1986; 186:300-302.
3. Gabrilove JL, Freiberg EK, Leiter E et al. Feminizing and non-feminizing Sertoli cell tumors. J Urol 1980; 124:757-767.
4. Zukerberg LR, Young RH, Scully RE. Sclerosing Sertoli cell tumor of the testis: a report of 10 cases. Am J Surg Pathol 1991; 15:829-834.
5. Kratzer SS, Ulbright TM, Talerman A et al. Large cell calcifying Sertoli-cell tumor of the testis: contrasting features of six malignant and six benign tumors and a review of the literature. Am J Surg Pathol 1997; 21:1271-1280.
6. Proppe KH, Scully RE. Large-cell calcifying Sertoli cell tumor of the testis. Am J Clin Pathol 1980; 74:607-619.
7. Young RH, Koelliker DD, Scully RE. Sertoli cell tumors of the testis, not otherwise specified. A clinicopathologic analysis of 60 cases. Am J Surg Pathol 1998; 22:709-7221.
8. Henley JD, Young RH, Ulbright TM. Malignant Sertoli cell tumors of the testis. A study of 13 examples of a neoplasm frequently misinterpreted as seminoma. Am J Surg Pathol 2002; 26:541-550.
9. McCluggage WG, Shanks JH, Whiteside C et al. Immunohistochemical study of testicular sex cord-stromal tumors, including staining with anti-inhibin antibody. Am J Surg Pathol 1998; 22:615-619.
10. Amin MB, Young RH, Scully RE. Immunohistochemical profile of Sertoli and Leydig cell tumors of the testis. Mod Pathol 1998; 11:76A.
11. Moran CA, Suster S. Yolk sac tumors of the mediastinum with prominent spindle cell features: a clinicopathologic study of three cases. Am J Surg Pathol 1997; 21:1173-1177.

QUESTIONS

1. Malignant Sertoli cell tumors are most often confused histologically with:
   - **A.** Granulosa cell tumor.
   - **B.** Metastatic melanoma.
   - **C.** Seminoma.
   - **D.** Metastatic carcinoma.
   - **E.** Benign embryological rests.
2. Which is the most likely immunohistochemical marker to be positive in malignant Sertoli cell tumor:
   - **A.** Smooth muscle actin.
   - **B.** S-100 protein.
   - **C.** Placental-like alkaline phosphatase.
   - **D.** Alpha-fetoprotein.
   - **E.** Inhibin

***Mark the appropriate letter.**

1. I tumori maligni a cellule di Sertoli sono per lo più istologicamente confusi con:
   - **A.** Tumore a cellule della granulosa.
   - **B.** Melanoma metastatico.
   - **C.** Seminoma.
   - **D.** Carcinoma metastatico.
   - **E.** Residui embriologici benigni.
2. Quale è il marker immunoistochimico a venire con più probabilità positivo nel tumore a cellule di Sertoli maligno?
   - **A.** Actina muscolare liscia.
   - **B.** Proteina S-100.
   - **C.** Fosfatasi alcalina "placental-like".
   - **D.** Alpha-fetoprotein.
   - **E.** Inibina.

***Contrassegnare la lettera giusta.**

CASE 4 25159-1

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CLINICAL HISTORY

A 57-year-old premenopausal female (gravida 2, para 2) presented with vaginal bleeding. An echoscan of the genital pelvic organs disclosed an anechoic left tubular tumor measuring 3 X 1 cm, along with two mural leiomyomas of the uterus. The patient underwent a bilateral salpingo-oophorectomy. Laboratory data showed no abnormality. The patient is well and free of disease at one year of follow-up.

PATHOLOGIC FINDINGS

Macroscopically, a 3-cm, solid intraluminal mass was noted in the left tube. Microscopically, the lesion was an exophytic neoplasm with a thin stalk continuing with the tubular epithelium. It was characterized by various proliferating patterns of tumor cells; glandular, tubular, sieve-like, solid, papillary and squamoid. Closely packed, confluent elongated glands were prominent. Some glands were composed of relatively uniform, columnar endometrioid type cells with mild atypia. Spindle cells growing in whorls indicated abortive squamous differentiation. The glands contained PAS-positive pinkish materials. The tumor cells had round, oval, or spindle nuclei with inconspicuous nucleoli and generally ill-defined, pale eosinophilic cytoplasm. Nuclear atypia was mild to moderate and mitotic activity was 10 /10HPF. Stroma was fibrous or edematous. There was no muscular invasion.

Special stains: The tumor cells were positive for CAM5.2, CK7, EMA, and CD10, but negative for inhibin.

DIAGNOSIS

Endometrioid carcinoma of the fallopian tube resembling a female adnexal tumor of probable Wolffian origin (FATWO).

DISCUSSION

Among primary Fallopian tube carcinomas, 71% were serous, 11% endometrioid, 8% transitional, 6% of mixed cell types, and 4% of miscellaneous other rare cell types. Daya and Young first reported wolffian-like endometrioid carcinoma of the fallopian tube. Twelve of 26 endometrioid carcinomas of the tube in a study by Alvardo-Cabrero et al. were Wolffian-like, characterized by a mostly solid proliferation of small, closely packed cells punctured by numerous small to cystic glands, imparting a superficial resemblance to female adnexal tumor of probable Wolffian tumor.

Main differential diagnosis includes FATWO, Sertoli-stroma cell tumor, granulosa cell tumor, and adenomatoid tumor. Several features favor the diagnosis of endometrioid carcinoma: 1) This tumor arises from the fallopian tube, FATWO typically arises outside the tube within the broad ligament or ovary. 2) The presence of squamous metaplasia or squamous differentiation is more in keeping with endometrioid carcinoma. 3) The degree of nuclear atypia and mitotic activity is more impressive than typically observed in FATWO. 4) Immunostains demonstrate EMA positivity and inhibin negativity. FATWO is positive for inhibin and negative for EMA. Sertoli-stroma cell tumor and granulosa cell tumor resembles histologically FATWO and

FATWO-like endometrioid carcinoma, but they can be ruled out because of the location and lack of squamous element. It must be aware that both Sertoli stromal cell tumor and granulosa cell tumor are inhibin (+), CAM 5.2 (+), CD99 (+), CD10 (-), and EMA (-) and that FATWO is positive for CD10. Adenomatoid tumor is characterized by crowded irregular spaces lined by flattened and cuboidal cells. It lacks squamous elements, cellular atypia, and mitotic figures. It is positive for cytokeratins, vimentin and calretinin and negative for EMA.
FATWO-like endometrioid carcinomas tended to present at very early stage like this case. Many of them had an growth limited to one tube without extension through or onto serosa. So, a prognosis of this type of tumor is far better than that of serous carcinoma and appears to be better than that of typical endometrioid carcinoma.

REFERENCES

1. Daya D, Young RH, Scully RE. Endometrioid carcinoma of the Fallopian tube resembling an adnexal tumor of probable Wolffian origin: a report of six cases. Int J Gynecol Pathol 1992; 11:122-130.
2. Kariminejad MH, Scully RE. Female adnexal tumor of probable wolffian origin. A distinctive entity. Cancer 1973; 31:671-77.
3. Devouassoux-Shisheboran M, Silver SA, Tavassoli FA. Wolffian adnexal tumor, so-called female adnexal tumor of probable Wolffian origin (FATWO): immunohistochemical evidence in support of a wolffian origin. 1999; 30:856-863.
4. Daya D. Malignant female adnexal tumor of probable wolffian origin with review of the literature. Arch Pathol Lab Med 1994; 118:310-312.
5. Williamson JMS, Armour A. Microcystic endometrioid carcinoma of the Fallopian tube simulating an adnexal tumour of probable Wolffian origin. Histopathology 1993; 23:578-880.
6. Alvardo-Cabrero I, Young RH, Vamvakas EC, Scully RE. Carcinoma of the fallopian tube: a clinicopathological study of 105 cases with observations on staging and prognosis factors. Gynecol Oncol 1999; 72:367-379.
7. Navani SS, Alvarado-Cabrero I, Young RH, Scully RE. Endometrioid carcinoma of the fallopian tube: a clinicopathologic analysis of 26 cases. Gynecol Oncol 1996; 63:371-378.

QUESTIONS

1. What is the commonest site of FATWO?
   Name the site: .....
2. Select two types of anti-body in order to differentiate FATWO-like endometrioid carcinoma from FATWO out of the following:
   - **A.** CAM5.2.
   - **B.** Vimentin.
   - **C.** EMA.
   - **D.** CD10.
   - **E.** Inhibin.

   ***For question 2 mark the appropriate letters.**

1. Quale è la sede più comune di insorgenza del FATWO (Tumore degli annessi uterini di origine wolffiana)?
   Nominare la sede: .....
2. Scegli due tipi di anticorpi per differenziare il carcinoma endometrioide "FATWO-like" dal vero "FATWO" tra quelli qui di seguito indicati:
   A. CAM 5.2.
   B. Vimentina.
   C. EMA.
   D. CD10.
   E. Inibina.
   ***Per la domanda 2 contrassegnare le lettere giuste.**

SURGICAL PATHOLOGY -SESSION II. UROGENITAL TRACT II

CASE PRESENTATIONS (4 cases)

Case 5. Uterine endometrioid carcinoma with small non-villous papillae

(*D. Ben-Dor*)

Case 6-I. Sertoli-Leydig cell tumor of the ovary of intermediate type, with low-grade heterologous gastrointestinal, retiform and questionable hepatoid components.

Case 6-II. Low grade Sertoli-cell tumor of the ovary in a centenarian woman

(*G. Falconieri*)

Case 7. Testicular tumor of the adrenogenital syndrome

(*M. Michal*)

Case 8. Lymphoepithelioma-like carcinoma of the renal pelvis

(*M. Fukunaga*)

CASE 5. 6903-99

David Ben Dor, M.D., Department of Pathology, The Barzilai Medical Center, Ashkelon, Israel.

CLINICAL AND PATHOLOGICAL PRESENTATION

This 64 year old woman who was at the time 10 years post menopause underwent endometrial curettage for "thick endometrium". No other history was given. The resulting fragments were diagnosed as low grade endometrioid adenocarcinoma. With that diagnosis hysterectomy was performed. Gross examination of the uterus revealed a leiomyoma and granular endometrium, without evidence of tumor masses. The cervix and adnexae were normal in appearance. Histology showed the bulk of the endometrium replaced by tumor with distinct papillary features. The prototype cell of this aspect was round with noticeable eosinophilic cytoplasm, containing a rounded nucleus with somewhat pale chromatin without a prominent nucleolus. These cells formed glands and detached round clumps in the lumen. Psammoma bodies were absent. Otherwise there was no noticeably increased mitotic activity or necrosis. There was no evidence of typical squamous differentiation. These features were most noticeable in the inner or superficial portion, while in the outer portion adjacent to the underlying myometrium there were atypical glands with typical endometrioid features: mostly columnar type cells, oval nuclei with coarser chromatin. The tumor was contained in the endometrium and did not invade into the myometrium. There was no tumor elsewhere in the specimen. The ovaries showed stromal hyperplasia.

The initial overall low power impression was that of a serous papillary tumor. These tumors in the uterus are by definition high grade, even if located superficially and of small dimension. However this tumor on close examination doesn't appear aggressive. Upon analysis the nuclei in a serous papillary carcinoma would be larger and more crowded, showing clearer chromatin and large distinct nucleoli. The nuclear cytoplasmic ratio would be much higher and the cytoplasm to the extent that it is seen is more distinctly basophilic. The appearance of the cells in this case is different as described above. Further there is a background of endometrial atypia which is not seen in serous papillary carcinoma of the endometrium. I was thus loath to assign a diagnosis of high grade malignancy on the basis of these findings, which would lead to aggressive therapy which might not be merited in this case.

The problem was in correctly applying the accepted diagnostic categories for endometrial neoplasms as listed in the fascicle on tumors of the uterus, third series, AFIP. Only two types of papillary tumors are admitted in the endometrium, serous papillary and villoglandular type. The distinction might at first be appreciated on low power examination. The latter shows thinner more filiform structures lined by endometrioid cells with distinctly low grade nuclei and is considered a type of endometrioid malignancy favored by estrogenic stimulation, unlike serous papillary carcinoma, which according to accepted conceptions is not hormonally driven and arises on the background of in situ carcinoma, not atypical hyperplasia. The pathologist is warned not to confuse these entities since the prognosis is so different. This case appeared to be a hybrid between these two entities based on morphologic considerations (i.e. small round shape seen in serous papillae but with low grade nuclei characteristic of the other type) and would be problematic as far as what the appropriate clinical approach should be.

I sent the case to Prof. Robert Young at Mass. General Hospital who was kind enough to review the slides and offer his opinion. He shared my observations and informed me that in fact he had collected and was in the process of studying a group of similar cases which he provisionally termed micropapillary endometrioid carcinoma. His formal final diagnosis of this case was low grade endometrioid carcinoma.

This case was received in 1999. Last year I examined another endometrial tumor which posed the same problems. I sent that case also to Prof Young who felt that it corresponded to the entity that he delineated and termed "uterine endometrioid carcinoma with small non-villous papillae" (replacing his earlier suggested term, which he seems to have abandoned so as not to be confounded with the nomenclature proposed by Kurman to describe a type of true serous tumors) in a recent article (1). This article describes 26 cases of endometrial neoplasms with the same features as the case I am discussing. Similar to this case, the published cases were typified by glands containing papillary buds lacking fibrovascular cores and composed of cells with ample eosinophilic cytoplasm and low n/c ratio (this element comprising at least 25% of the tumor, which could otherwise show classical endometrioid features, with or without villoglandular type papillae). Squamous differentiation was seen in 50% of cases. Nuclear atypia was grade 1 or 2 (of 3) and the FIGO grade was 1-2. Whereas the majority (21/26) showed myometrial invasion, it was mostly limited to the inner half of the myometrium. Only rare cases showed lymph node or bone metastases. Overall the behavior of this tumor is much more in keeping with that of typical endometrioid carcinomas than the aggressive behavior of serous papillary cancer. These cases constituted about 7% of the total number of cases of endometrial carcinoma seen at Mass General Hospital over the five year period reviewed, actually being more prevalent than the number of cases of true serous papillary carcinoma in the endometrium seen over the same period. The average age of the patients was in between that patients with typical endometrioid adenocarcinoma and those with endometrial serous papillary carcinoma. The authors found in reviewing numerous articles and texts that this type of histology has not been previously acknowledged in an organized or systemic fashion.

DIAGNOSIS

Uterine endometrioid carcinoma with small nonvillous papillae.

DISCUSSION

The purpose of this presentation has been to demonstrate a recently described variant of endometrioid carcinoma which bears superficial similarities to serous papillary tumors and thus may be confounded with it. It is important to recognize it so as not to automatically brand all endometrial carcinomas with small round papillae as serous papillary tumors, since the latter have a worse prognosis than the cases described here. In the Mass General material summarized in the cited article this type of tumor is actually slightly more prevalent than serous papillary carcinomas of the uterus, despite the latter having invited much more attention. It is interesting to speculate how a case such as this would have been diagnosed in the past. Actually, in the modern literature, papillary tumors of the endometrium have been receiving attention only since the latter part of the seventies; the notion of serous papillary carcinoma as a distinct high grade neoplasm in the endometrium was introduced in 1982 (2). References to the villoglandular form appear slightly afterwards (3). In fact, my diagnosis of the preliminary curettage in this case was low grade endometrioid carcinoma, apparently having ignored the papillary component. Since these tumors often appear on the background of an endometrioid process, it is possible that

many were assimilated to the more familiar category. In the years following the recognition of papillary differentiation in endometrial tumors, villoglandular endometrioid carcinoma would be an appropriate alternative at least biologically. The danger would be in mistaking this type of tumor for its high grade relation (serous papillary carcinoma) which could result in inappropriate treatment. Hopefully the coming to light of this entity would give the aware pathologist the appropriate category for this tumor.

The subject of papillary endometrial lesions was taken up again in an article which appeared most recently (4). In these cases the papillae were not deemed malignant, all showed fibrovascular cores, and were covered by metaplastic cells. This finding casts new light on the rule that all papillary lesions in the endometrium are malignant. The article also brings up the point that ordinary epithelial metaplastic phenomena can show tufts, buds, and micropapillae. This is seemingly illustrated elsewhere in standard references (see fig. 11.26, p. 428 in (5); also see fig. 7-20, p. 191 and 7-21, p. 192 in (6). These phenomena might illustrate benign versions of the differentiation shown by the type of tumor in this case. It should also be pointed out that the phenomenon termed syncytial metaplasia is actually a degenerative/regenerative process and not truly neoplastic.

In summary, the different types of papillary carcinomas in the endometrium have been discussed here, with the presentation of a recently described entity, endometrioid carcinoma with small non villous papillae. The importance of recognizing this is not to confound it with the high grade serous papillary carcinoma. One may envision two morphological types of papillae, short round ("serous") and thin (villous) each one having low-intermediate and high-grade variants. Up until now, the serous type was considered to be high grade generically, the villous type to be low grade. The potential of a low grade tumor with a "serous" appearance has now been fulfilled with the identification of cases such as the one demonstrated here. (Parenthetically these should not be regarded as low grade serous carcinomas, as serous carcinomas are classified as non-endometrioid whereas this type of tumor usually develops from an endometrioid background). What is left are high grade villous papillary tumors of aggressive potential. In my own practice I have in fact begun to notice high grade endometrial carcinomas with at least focally a component of thin delicate papillae lined by high grade malignant cells. Thus this type of differentiation is possible and it remains to be seen if pathologists will begin to notice tumors in which it occupies a significant proportion of the tumor, thus deserving a subcategory of its own.

POSTSCRIPT: the index patient presented here has not shown recurrence of the tumor.

REFERENCES

1. Murray S.K. et al. Uterine endometrioid carcinoma with small nonvillous papillae: an analysis of 26 cases of a favorable-prognosis tumor to be distinguished from serous carcinoma. Int J Surg Pathol 2000; 8: 279-289.
2. Hendrickson M. et al. Uterine serous papillary carcinoma: a highly malignant form of endometrial adenocarcinoma. Am J Surg Pathol 1982; 6: 93-108.
3. Chen J.L. et al. Endometrial papillary carcinoma: two clinicopathological types. Int J Gynecol Pathol 1985; 4: 279-288.
4. Lehman M. and Hart W. Simple and complex hyperplastic papillary proliferations of the endometrium. Am J Surg Pathol 2001; 25: 1347-1354.

5. Kurman R., ed. Blaustein's Pathology of the Female Genital Tract, 4th ed. New York: Springer Verlag, 1994.
6. Hendrickson M. and Kempson R. Surgical Pathology of the Uterine Corpus (vol. 12, Major Problems in Pathology). Philadelphia: W. B. Saunders, 1980.

QUESTIONS assessment

1. Papillary tumors of the endometrium behave in the same manner.
   True or False. _____.
2. Correct assessment of papillary tumors in the endometrium always requires cytologic evaluation of the neoplastic cells. **True or False.** _____.

1. I tumori papillari dell'endometrio si comportano tutti nello stesso modo.
   Vero o Falso. _____.
2. La valutazione corretta dei tumori papillari dell'endometrio richiede sempre una valutazione citologica della neoplasia. **Vero o Falso.** _____.

CASE 6I & CASE 6II

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CASE 6I. 01-3731

CLINICAL HISTORY

A 17 year-old girl was admitted to the emergency room for a few day history of abdominal pain and discomfort. Past medical history was unremarkable. She denied endocrine disturbances although on physical inspection she appeared slightly obese and hirsute. Menstrual history was referred as normal. On admission she was non-febrile and said that the pain had worsened during the previous hours. An ultrasound scan revealed a multiloculated solid mass almost filling the entire abdominal cavity. Preoperative laboratory investigations showed an abnormal alfa-fetoprotein serum concentration (2047 UI, upper normal limit 10). The Ca125 (392 UI, upper normal limit 35) and Ca 19.9 serum levels (92 UI, upper normal limit 39) were markedly elevated as well. At laparotomy, a left sided ovarian mass was found. Unilateral salpingo-oophoreectomy was done. Following surgery, the abnormal serum markers returned within the normal limits.

PATHOLOGY

GROSS FINDINGS

The specimen measured 30 x 16 x 12 cm and weighed 10 kg. The external surface was tense, grey, with bulging bluish areas corresponding to sub-capsular, larger cystic spaces. Cut section showed a multiloculated tumor with thick, grey-white rubbery septa delimiting variably shaped cavities filled with jelly-like, sometimes granular material. The cyst lumina were covered by soft, glistening polypoid formations.

HISTOLOGIC FINDINGS

Microscopic examination of HE stained section showed that the cysts were lined up by glandular epithelium resembling that of gastric foveolae or colonic mucosa. Goblet cells were clearly recognizable. The intervening stroma was loosely textured and featured cords and nests of small hyperchomatic cells admixed with larger polygonal cells having eosinophilic cytoplasm. Paucicellular aggregates as well as larger cellular sheets were present. Some hollow tubules covered by small hyperchromatic and flattened cells were also scattered within the fibrous stroma; cystic formation with small papillary protrusions or epithelial bridges, imparting a retiform pattern was consistently noted throughout. Mild to moderate atypia was observed in the cell nests; mitotic figures, although in a low count, were unequivocally present in the more cellular areas. The mucinous epithelium was uniformly bland, however nuclear stratification, loss of polarity and some nucleolar prominency could be focally recognized. Some cystic spaces contained a pink homogeneous "thyroglobulin-like" material.

IMMUNOHISTOCHEMICAL PROFILE

The well differentiated gastrointestinal epithelium reacted for antibodies against broad spectrum keratins, EMA and carcinoembryonic antigen; occasional small cells intercalated within the surface gland elements were immunopositive for serotonin and synaptophysin. Epithelial cells were negative for vimentin, AFP, ER, PR, S100 protein. The small and larger cells scattered within the stroma exhibited a complementary immunopositivity for vimentin. Small cells were also focally positive for broad spectrum keratins but not for

EMA. Scattered, polygonal cells phenotypically corresponding with the larger cells with pink granular cytoplasm were strongly positive for keratins and focally, but unequivocally, for AFP. Other stains including S100 protein, alfa-fetoprotein, desmin, neurofilaments, ER, PR were negative. was positive in the specialized small and large stromal cells.

DIAGNOSIS

Sertoli-Leydig cell tumor (SLCT) of intermediate type, with low-grade heterologous gastrointestinal, retiform and questionable hepatoid components.

DISCUSSION

SLCT of the ovary account for less than 0.5% of ovarian neoplasms.[13] Although they may occur at any age, most cases are encountered during the second-third decades of life with an average patient age of 25 years. Symptoms related to sex-hormone productions may be present; virilization being noted in as much as half patients. Hirsutism, voice deepening and clitoral enlargement are the most frequently noted abnormalities. In a minor percent of cases signs related to hyperestrogenism may occur. SLCT may vary in their gross aspect, however tumors having a mucinous component may attain a huge size. [17] Necrosis and hemorrhage are encountered in high-grade lesions. According to the WHO classification SLCT segregate as follows[13]:

1. well differentiated tumors, featuring hollow or solid tubules similar to those described for Sertoli cell tumor. The intervening stroma contains number of cells consistent with Leydig cells. The stromal component consists of a relatively inconspicuous amount of mature fibrous tissue. Hyalinization may be occasionally present with small tubules scattered within a pink, fibrous rich ground substance.
2. intermediately differentiated tumors, showing cellular areas alternating with hypocellular or edematous connective tissue. Nests, small clusters and cords of cells compatible with immature Sertoli cells are present along to cells or nests of cells with abundant, stainable cytoplasm which represent Leydig cells. Nuclei with prominent nucleoli can be recognized among this population. Hollow tubules comparable to those of well differentiated tumors may still be present. Large sheet-like aggregates of Leydig cells can be present. A moderate mitotic activity is common
3. poorly differentiated tumors, showing predominantly spindle and/or small round cells, simulating a poorly differentiated carcinoma. The distinctive SLCT features can be obscured and are often demonstrable only after thorough lesion sampling

About 20% of SLCT feature a heterologous component mainly consisting of mucinous epithelium of gastrointestinal type. [18,20] Approximately 10% of cases, usually of the intermediate and poorly differentiated types, may have microscopic formations simulating the rete of the testis, i.e. slit-like tubules lined up by cuboidal cells with scant cytoplasm and small nuclei, hence the retiform appellation coined by dr Scully and associates in 1982[10,19]. These tumors may feature cystic transformation of tubules, and small papillary projections similar to those seen in epithelial serous tumors can be seen.

As mentioned, the most common component seen in heterologous tumor is mucinous epithelium of gastrointestinal type.[18] The latter is fully comparable to that lining the gastric foveolae or the intestinal mucosa. In a minor but sizable percent of cases, argentaffin cells may be recognized, recapitulating the phenotypic and immunohistochemical characteristic of neuroendocrine gastrointestinal cells (individual or small nests of small polygonal elements with central nuclei and scant granular cytoplasm, positivity for some markers

such as chromogranin, serotonin and synaptophysin). Nests of carcinoids may occur in about half cases although they are easily overlooked because of their small size.[16]
The mucinous epithelium of heterologous SLCT is generally benign, however in some cases, as the present appears to be, it is of borderline or low-grade type. At present, there is no evidence that mucinous borderline or carcinoid tumors have a significant impact on the prognosis of SLCT, which still correlates to the grade of the stromal proliferation.[16] Nevertheless, the mucinous component bears significant diagnostic importance since it can mimic a pure epithelial tumor, especially when it is so exuberant as to obscure the stromal proliferation. The mucinous component accounts for the large size attained by these tumors which, along to the abnormal serum markers detected preoperatively, may suggest a true borderline or invasive cystadenoarcinoma.
Less commonly, heterologous SLCT may contain non-epithelial elements such as cartilage, immature skeletal muscle, or bone.[8,12,18] These tumors tend to have more hemorrhagic and necrotic areas on gross inspection and are often composed of atypical, sarcomatoid elements, and generally have a poor clinical outcome.
In a minor percent of cases SLCT may have a focal component of so called "hepatoid cells" (large polygonal cells with granular cytoplasm, vesicular nucleous with a prominent nucleolus); these tumors are likely to be associated with high serum levels of AFP.[6,17] High serum levels of AFP may be observed also in SLCT without microscopic evidence of hepatoid differentiation especially in tumors overwhelmed by conspicuous heterologous mucinous or retiform components.[3,9] Our case may well be one of these tumors perhaps having a focal hepatoid component buried within it that skipped to extensive tumoral sampling.
Recently, molecular pathology techniques has cast doubt about the truly neoplastic nature of the Leydig cell component of SLCT.[7] In particular, the view they may represent a subset of reactive "bystanders" is supported by the demonstration of a low proliferative fraction and loss of heterozygosity.

DIFFERENTIAL DIAGNOSIS

SLCT are rare and since they have a wide range of microscopic patterns they can be confused with numerous ovarian tumors, either of epithelial or stromal types.
Well differentiated endometrioid carcinoma can simulate a SLCT and the distinction may be difficult as long as it features small glands or solid tubular aggregates with minimal nuclear atypia and a low mitotic count.[1,11] However, endometrioid carcinomas have usually residual areas of tumor, with larger and more irregular glands. Endometrioid carcinoma is positive for keratins and epithelial membrane antigen, whereas SLCT, albeit reportedly positive for keratins, is negative for EMA. In contrast, inhibin is positive in SCLT.[1,4,5] The mucinous heterologous component of SLCT may be totally indistinguishable from a low-grade (borderline) or invasive mucinous adenocarcinoma; the latter may be occasionally associated with masculinization, thus making the distinction difficult on a clinical basis only. Furthermore, both the lesion may share a conspicuous sizing, especially if the SLCT has a preponderant heterologous gastrointestinal component. However, epithelial tumors are more common in the perimenopausal age and uncommon in the first two decades of life, when stromal tumors have their peak frequency.[16,20] The correct interpretation rests on microscopic recognition of Sertoli-Leydig cell neoplasia, which are usually present in the stroma between the gland and the cysts. This may require extensive lesion sampling especially in larger tumors. The differential diagnosis with endodermal sinus tumor or yolk sac tumor deserves a special attention. In fact, some microscopic features of retiform SLCT such as the slit-like tubules, papillae and even abortive glomeruloid structures recalling the Schiller-Duvall bodies, are apt to be confused with the reticular pattern of

endodermal sinus tumor.[10] The latter however, in addition to a gross malignant appearance, has a distinctive reticulated or micropolycistic configuration of tumor cells. Moreover, endodermal sinus tumor has clear cut features of malignancy such as irregular nuclei with prominent nucleoli and a high mitotic rate. Androgenic manifestations, common in SLCT, are unlikely in yolk sac tumors. AFP immunopositivity of yolk sac tumors helps in the differential diagnosis, yet it may be encountered in SLCT especially in cases having an hepatoid component. Retiform SLCT exhibiting a preponderant papillary pattern and cellular stratification over the papillae may be confused with serous papillary carcinoma. Again, a variety of clinical and pathological features are helpful clues, since serous tumors occur in aged women, are often bilateral, and are rarely if not exceptionally associated with virilization. Extra-ovarian spread is much more frequent with epithelial tumors than SLCT. On a microscopic basis, the presence of more easily recognizable patterns of SLCT enables the correct interpretation.
Finally, SLCT with heterologous gastrointestinal type epithelium have to be distinguished by a metastatic mucinous carcinoma (krukenberg tumor) from a primary outside the ovary. Tubular krukenberg tumors may imitate histologically SLCT especially if luteinization is present. Clinical history is crucial to the correct interpretation. Krukenberg tumor is generally bilateral and has markedly atypical cells, including signet ring cells containing mucin.

CASE 6II 01-13724

CLINICAL HISTORY

A 103 year-old woman complained of pain and micturition disturbances including frequency and urine loss. On palpation of the right iliac fossa a firm tenderness adnexal mass was noticed. An ultrasound scan confirmed the presence of a solid pelvic lesion. Exploratomy laparotomy was done and the mass resected.

PATHOLOGY

GROSS FEATURES

The gross specimen consisted of a solid, lobulated mass measuring 18 cm in largest dimension. The cut surface was pink yellow and homogeneous except for some areas of tissue softening.

MICROSCOPIC FEATURES

The tumor is uniformly composed of solid tubules surrounded by a delicate rim of fibroconnective tissue. The latter had inconspicuous stromal cells usually of fibroblastic type. Lumina were inconspicuous and, when present, of punched out type. The tubules were composed of columnar cells featuring clear to slightly tingible cytoplasm. Nuclei were round to oval with evenly distributed chromatin and a low to moderate mitotic activity (1-3 per 10 HPF).

IMMUNOHISTOCHEMISTRY

Tumor cells were negative for epithelial markers including keratins (broad spectrum AE1/3, Cam 5.2, Ker 5/6, 34BE12) and epithelial membrane antigen. Synaptophysin, chromogranin, serotonin, HMB45 and S100 protein were negative as well. Strong immunoreactivity for vimentin was noted. Strong immunoreaction for inhibin was also documented. Focal positivity for melan A and calretinin was also documented. The proliferation rate evaluated by means of the Mib-1 (ki-67) antibody was 30%.

DIAGNOSIS

Low grade Sertoli-cell tumor.

DISCUSSION

Sex cord-stromal tumors of the ovary encompass a broad category of histologically diverse lesions derived from the specialized ovarian stroma. They account approximately for 6% of ovarian tumors and affect primarily women in the reproduction age. Hormonally active neoplasms (producing either virilizing or hyperestrogenic) are often seen although non-functioning types are equally common.[13] SLC are rare and usually occurs in young woman; in more than half cases there are symptoms referred to hyper-estrogenism. Isosexual precocious puberty has been occasionally reported. The tumors are unilateral and confined to the ovary. Grossly, they are solid and average 9 cm in larger dimension. Cut surface shows a typical yellow-grey surface with a lobular outline. Necrotic changes may occur in larger lesions. Microscopically, SLC may feature either empty hollow tubules, i.e. uniform, small, round tubular formation provided with punched out borders, or solid tubules consisting in round, oval cells that can simulate the prepubertal testis or the atrophic seminiferous tubules seen in the adult males. Hollow tubules may contain occasionally some pink, granular secretion. The cells may have moderate cytoplasm with clear spaces probably due to lipid droplets lost during tissue processing. The nuclei usually lack atypical features but mitotic activity may be present. Tumor cells are associated with scant fibrous stroma, with usually little or no intervening cells other than scarce fibrocytes. At times, scattered Leydig cells may be present. [13] The clinical course is generally benign, although exceptional cases of Sertoli cell carcinomas have been published.[21] Immunohistochemically, SCT are positive for vimentin and generally negative for epithelial markers. Inhibin is reliably positive and may be used in dubious cases.[1,4,5] Combined positivity for calretinin and Melan A, consistently reported in SCT, is useful although not entirely discriminatory for distinguishing these tumors from other lesions.[2,14]

DIFFERENTIAL DIAGNOSIS

This case poses more problems from the clinical rather than pathologic point of view. If observed in young-adult woman with symptoms related to estrogen production, as usually happens with most stromal tumors of ovary, we would not have so much hesitation in calling it SCT merely based on ordinary HE stained slides. As a matter of fact, the occurrence in a centenarian obligates us to exclude first those entities more frequently seen in elderly women.

Sometimes endometrial carcinoma may have a pattern closely comparable to a SCT.[11] However, conventional area of carcinomatous differentiation such as larger and irregular glands are generally present, often along to areas of squamoid differentiation. The latter are typically absent in SCT. A metastatic carcinoma (krukenberg tumor) may be excluded in virtue of the clinical history, microscopic pattern (lack of markedly atypical cells; absence of glandular differentiation, immunonegativity of EMA) and bilaterality. Carcinoid tumors are apt to be confused with SCT having solid tubules, especially when the ribbons and nests of tumor cells retract from the adjacent supporting stroma. Cell nuclei may display some fine distribution of the chromatin texture and the cell cytoplasm contain granular material. However, SCT are negative for EMA and does not stain for neuroendocrine markers. Also, SCT features a less fibromatous stroma when compared to carcinoid. It should be emphasized that keratin immunoreactivity is often observed in stromal tumors of the ovary, including granulosa cell tumors, SCT and SLCT. Rather, EMA immunostaining is more meaningful in excluding a stromal tumor, regardless of keratin immunoreactivity.

Ovarian tumors of wolffian origin may have a prominent tubular pattern and are thus easy to confound with SCT. Nevertheless, SCT rarely has the admixture of other patterns including sieve-like and solid areas composed of small oval or spindle shaped cells, as is customarily observed in tumors of wolffian origin. Sclerosing stromal tumors occurs usually in younger patients (average in the 3-4th decade) however cases in postmenopausal ages have been reported. [13] They may have microscopic areas reminiscent of SCT (nests of polygonal cells with clear cytoplasm surrounded by delicate fibrous tissue). However, sclerosing stromal tumor has a characteristic gross presentation (a yellow rubbery mass due to increased fibrous substance imparting peripheral lobulation to the cut surface and scarring in the central portion) and microscopically the typical pattern of thick fibrous bands delimiting myxoid, low cellular areas can be seen. During the last few years, several cases of pelvic tumors in women have been reported featuring perivascular epithelioid cells arranged in nests delimited by scant hyalinized stroma; such tumors, recently devised as perivascular epithelioid cell tumors or PEComas, may mimic quite closely SCT. [15] Both Pecomas and SCT may be immunopositive for melan A and calretinin, [2,15] however Pecomas may show a variable immunoreactivity for S100 protein and HMB-45, which are conversely lacking in SCT.

REFERENCES

1. Aguirre P, Thor, Scully RE. Ovarian endometrioid carcinomas resembling sex-cord-stromal tumors. An immunohistochemical study. In J Gynecol Pathol 1989; 8: 364-373
2. Cao QJ, Jones LG, Li M. Expression of calretinin in human ovary, testis and ovarian sex cord-stromal tumors. Int J Gynecol Pathol 2001; 20: 346-352
3. Hammad A, Jasnosz KM, Olson PR. Expression of alpha-fetoprotein by ovarian Sertoli-Leydig cell tumors. Case report and review of the literature. Arch Pathol Lab Med 1995; 119: 1075-1079
4. Kommos F, Oliva E, Bhan AK, Scully RE. Inhibin expression in ovarian tumors and tumor-like lesions. Mod Pathol 1998; 11: 656-664
5. Kommos F, Oliva E, Bhan AK, Young RH, Scully RE. Inhibin expression in ovarian tumors and tumor-like lesions: An immunohistochemical study. Mod Pathol 1998; 11: 656-664
6. Mooney EE , Nogales FF, Tavassoli FA. Hepatocytic differentiation in retiform Sertoli-Leydig cell tumors: distinguishing a heterologous element from Leydig cells. Hum Pathol 1999; 30: 611-617
7. Mooney EE, Man YG, Bratthauer GL, Tavassoli FA. Evidence that Leydig cells in Sertoli-Leydig cell tumors are reactive rather than neoplastic. Cancer 1999; 86: 2312-2319
8. Mooney EE, Vaidya KP, Tavassoli FA. Ossifying well differentiated Sertoli-Leydig cell tumor of the ovary. Ann Diagn Pathol 2000; 4: 34-38
9. Motoyama I, Watanabe H, Gotoh A, Takeuchi S, Tanabe N, Nashimoto I. Ovarian Sertoli-Leydig cell tumor with elevated serum alpha fetoprotein. Cancer 1989; 63: 2047-2053
10. Moyles K, Chan YF, Hamill J, Massey R. Ovarian Sertoli-Leydig cell tumor with retiform pattern. Pathology 1995; 27: 371-373
11. Ordi J, Schammel DP, Rasek L, Tavassoli FA. Sertoliform endometrioid carcinomas of the ovary. A clinicopathologic and immunohistochemical study of 13 cases. Mod Pathol 1999; 12: 933-940
12. Prat J, Young RH, Scully RE. Ovarian Sertoli-Leydig cell tumors with heterologous elements. II. Cartilage and skeletal muscle. A clinicopathologic analysis of twelve cases. Cancer 1982; 50: 2465-2475

13. Scully RE, Young RH, Clement PB. Tumors of the Ovary, Maldeveloped Gonads, Fallopian Tubes, and Broad Ligament. Armed Forces Institute of Pathology, Washington, 1998
14. Stewart CJ, Nandini CL, Richmond JA. Value of A103 (melan-A) immunostaining in the differential diagnosis of ovarian sex cord stromal tumours. J Clin Pathol 2000; 53: 206-211
15. Vang R, Kempson RL. Perivascular epithelioid cell tumor (PEComa) of the uterus. Am J Surg Pathol 2002; 26: 1-13
16. Young RH, Bell DA, Clement PB. Recent advances in the pathology of ovarian tumors. Mod Pathol 1995; 8: 930-959
17. Young RH, Perez-Atayde AR, Scully RE. Ovarian Sertoli-Leydig cell tumors with a retiform pattern and heterologous component. Report of a case with hepatocytic differentiation and elevated serum alfa-fetoprotein Am J Surg Pathol 1984; 8: 709-718
18. Young RH, Prat J, Scully RE. Ovarian Sertoli-Leydig cell tumors with heterologous elements. I. Gastrointestinal epithelium and cartilage. A clinicopathologic analysis of thirty-six cases. Cancer 1982; 50: 2448-2456
19. Young RH, Scully RE. Ovarian Sertoli-Leydig cell tumors with a retiform pattern. A problem in histopathologic diagnosis. A report of 25 cases. Am J Surg Pathol 1983; 7: 755-771
20. Young RH, Scully RE. Ovarian Sertoli-Leydig cell tumors. A clinicopathologic analysis of 207 cases. Am J Surg Pathol 1985; 9: 543-569
21. Young RH, Scully RE. Ovarian Sertoli cell tumors. A report of 10 cases. Int J Gynecol Pathol 1984; 2: 349-363

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QUESTIONS

1. Which of the following is the single BEST clinical or pathological parameter in the diagnostic approach to ovarian tumors?
   A. Serum concentration of AFP
   B. Patient's age
   C. History of abnormal uterine bleeding
   D. Tumor stage at presentation
   E. Size of tumor
2. Which of the following statements regarding ovarian tumors is INCORRECT:
   A. Mucinous tumors are amongst the larger ovarian neoplasms
   B. Epithelial tumors are rarely associated with endocrine abnormalities
   C. Sertoli-Leydig cell tumors have often a poor prognosis
   D. Serous carcinomas are frequently bilateral at presentation
   E. Leydig cell tumors are usually seen in pre-menopausal age

***Mark the <u>best</u> answer, not just a good answer!**

1. Quale è il miglior parametro clinico-patologico nell'approccio diagnostico ai tumori ovarici?
   - **A.** Concentrazione sierica di Alfa-feto-proteina.
   - **B.** Età del paziente.
   - **C.** Storia di perdite ematiche vaginale anomale.
   - **D.** Stadio del tumore alla diagnosi.
   - **E.** Dimensioni del tumore.
2. Quale delle seguenti affermazioni concernenti i tumori ovarici è errata:
   - **A.** I tumori mucinosi sono tra le neoplasie ovariche più voluminose.
   - **B.** I tumori epiteliali sono raramente associati con disturbi endocrini.
   - **C.** I tumori a cellule di Sertoli-Leydig hanno spesso una cattiva prognosi.
   - **D.** I carcinoma sierosi sono spesso bilaterali al momento della diagnosi.
   - **E.** I tumori a cellule di Leydig si osservano di solito in età pre-menopausale.

***Contrassegnare la migliore risposta, e non già solo una buona risposta, tra quelle date alle domande formulate!**

CASE 7. M14164/97

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CLINICAL HISTORY

Bilateral tumor of the testes in a 14 year-old patient. The blocks are taken from the left testis. The lesion was composed of several nodules which were up to 2,5 cm in size.

DIAGNOSIS

Tumor of the adrenogenital syndrome of the testis (TTAS).

COMMENT

TTAS typically arise in testis in patients with adrenogenital syndrome. The adrenogenital syndrome is of the "salt-losing form" in two thirds of the patients with TTAS. The lesions are usually bilateral with infrequently additional masses found in along the spermatic cord. Histologically the TTAS can be mistaken with Leydig cell tumor (1,5,6,8). Histologically TTAS, however, differs slightly from bilateral Leydig cell tumor. It is always divided by fibrous tissue into the lobules, if often shows lipid rich change of the cytoplasm and lipofuscin pigment. It always lacks Reinke's crystals. Often a golden-brown lipochrome pigment can be seen in the cytoplasm of the cells. Nuclei can be hyperchromatic. A diagnostic pitfall in these tumors are nodular proliferations of the interstitial cells containing the Reinke's crystals which might be mistaken for the part of the tumor with inferable diagnosis of bilateral Leydig cell tumor.

TTAS is not an autonomous neoplasm but it is dependent on the elevated levels of ACTH that occur in the adrenocortical syndrome. The size of TTAS usually decreases after the administration of the corticosteroids (4).

Sometimes identically looking tumor appears within the Nelson's syndrome (the development of an ACTH secreting pituitary adenoma after bilateral adrenalectomy for Cushing's syndrome) (3,7). Very rarely similar tumor mass can be seen in the ovaries in patients with adrenogenital syndromes (1).

REFERENCES

1. Al-Ahmadie HK, Stanek J, Liu J, Mangu PN, Niemann T, Young RH. Ovarian „tumor" of the adrenogenital syndrome. The first reported case. Am J Surg Pathol 2001:25:1443-1450
2. Cunnah C, Perry L, Dacie JA, Grant DB, Lowe DG, Savage MO, Besser GM. Bilateral testicular tumours in congenital adrenal hyperplasia: a continuing diagnostic and therapeutic dilemma. Clin Endocrin 1989:30:141-147
3. Krieger DT, Samojlik E, Bardin CW. Cortisol and androgen secretion in a case of Nelson's syndrome with paratesticular tumors: response to cyproheptadine therapy. J Clin Endocrin Met 1978:47:8737-844
4. Prader A. Die Cortisondauerbehandlung des Kongenitalen Adrenogenitalen Syndroms. Helv Paediat Acta 1953:8:386-423
5. Rutgers JL, Young RH, Scully RE. The testicular "tumor" of the adrenogenital syndrome. Am J Surg Pathol 1988:12:503-513
6. Radfar N, Bartter F.C, Easley R, Kolins J, Javadpour N, Sherins RJ. Evidence for endogenous LH suppression in a man with bilateral testicular tumors and congenital adrenal hyperplasia. J Clin Endocrin Met 1977:45:1194-1204

7. Rosenzweig JL, Lawrence DA, Vogel DL, Costa J, Gorden P. Adrenocorticotropin independent hypercortisolemia and testicular tumors in a patient with a pituitary tumor and gigantism. J Clin Endocrin Met 1982:55:421-427
8. Shanklin DR, Richardson AP, Rothstein G. Testicular hilar nodules in adrenogenital syndrome. The nature of the nodules. Am J Dis Child 1963:106:243-250

QUESTIONS

1. Are these tumors always bilateral? **Yes or No.** _____.
2. Does the tumor of the adrenogenital syndrome have Reinke's crystals? **Yes or No.** _____.

1. Questi tumori sono sempre bilaterali? **Si o No.** ____.
2. Questo tumore, proprio della sindrome adrenogenitale, presenta i cristalli di Reinke? **Si o No.** ____.

CASE 8 S90-1597

Masaharu Fukunaga M. D., Department of Pathology, Jikei University, School of Medicine, The Daisan hospital, Izumi-honcho, Komaeshi, Japan.

CLINICAL HISTORY

A 70-year-old Japanese male presented with a 20-month history of hematuria. He had no history of malignancy. CT scan, descending IVP, and renal angiogram revealed a mass in the pelvis of the left kidney. The patient underwent an abdominal nephroureterectomy with postoperative radiotherapy (50 Gy). Follow-up studies, including ENT examination, failed to demonstrate occult malignancy. The patient was well with neither recurrence nor metastasis 6 years after the diagnosis.

PATHOLOGY

Macroscopically, a 5 X 3 X 3 cm, well-demarcated, white, soft, partially papillary tumor was found in the pelvis of the kidney. Histologically, it was characterized by a syncytial arrangement of undifferentiated malignant cells and a dense lymphoplasmacytic infiltrate (predominantly UCHL-1 positive lymphocytes). The tumor was limited in the pelvis with a minute focus of carcinoma in situ (your slide may not contain it, my apologies). The tumor cells were positive for CAM5.2, CK 7, CK 19, CK 20, EMA, and Leu M1. No Epstein-Barr viral genomic sequences were detected by ISH. The tumor had an aneuploid DNA content.

DIAGNOSIS

Lymphoepithelioma-like carcinoma (LELC) of the renal pelvis

COMMENT

The tumor is very similar to lymphoepithelioma of the nasopharynx. This case, to the best of my knowledge, may be the first case of LELC of the renal pelvis (1). Although the tumor cells did not resemble light-microscopically transitional epithelium, the profiles of CK 7+/ 20+ in the tumor were the same as those of transitional cell carcinoma) of the urinary bladder and renal pelvis, indicating urothelial differentiation. The EBV genome was not found within the tumor, in either neoplastic cells or lymphocytes by ISH (nasopharyngeal lymphoepitheliomas are always positive for EBV genome). It is not clear whether the method used in this case was not sensitive enough to detect the existence of the viral genome or whether this lesion is not associated with EBV. The follow-up study indicates favorable outcome of the neoplasm, probably due in part to the strong host immune response mounted against the tumor cells. All of the nine patients with LELC of the urinary bladder have also had favorable clinical courses without local or distant recurrence (2-4). This type of carcinoma in general has an extremely good response to radiotherapy. Recognition of this type of the renal pelvis is important to avoid misdiagnosis and to choose appropriate treatments.

REFERENCES

1. Fukunaga M, Ushigome S. Lymphoepithelioma-like carcinoma of the renal pelvis: A case report with immunohistochemical analysis and in situ hybridization for Epstein-Barr viral genome. Mod Pathol 1998; 11: 1252-1256.

3. Amin MB, Ro JY, Lee KM, et al. Lymphoepithelioma-like carcinoma of the urinary bladder. Am J Surg Pathol 1994; 18:466-473.
4. Young RH, Eble JN. Lymphoepithelioma-like carcinoma of the urinary bladder. J Urol Pathol 1993;58:63-67.

QUESTIONS

1. Are lymphoepithelioma-like carcinomas radiosensitive? **Yes or No.** ____.
2. Have all extra nasopharyngeal lymphoepithelioma-like carcinomas been proved to be associated with Epstein-Barr virus? **Yes or No.** ____.

1. I carcinomi "lymphoepitelioma-like" sono radio-sensibili? **Si o No.** ____.
2. Tutti i carcinomi "lymphoepithelioma-like" extra-nasofaringei si sono dimostrati associati con virus di Epstein-Barr? **Si o No.** ____.

SURGICAL PATHOLOGY –SESSION III. SOFT TISSUE & BONE

CASE PRESENTATIONS (5 CASES)

Case 9. Biphasic spindle and epithelioid myoepithelioma of soft tissue, oncocytic type

(M. Bisceglia)

Case 10. Acral myxoinflammatory tumor of soft tissue

(*K. Cooper*)

Case 11. Clear cell chondrosarcoma of bone

(*J. Lamovec*)

Case 12. Low-grade fibromyxoid sarcoma of soft tissue

(*M. Fukunaga*)

Case 13. Inflammatory leiomyosarcoma of bone

(*D. Ben-Dor*)

CASE 9. 100083-99

Michele Bisceglia, M.D., Department of Pathology, IRCCS-Hospital "Casa Sollievo della Sofferenza", San Giovanni Rotondo (FG), Italy.

CLINICAL HISTORY

A 58-year old female patient presented with a large tumor of the anterior chest wall, involving the soft tissues and the periosteum of her upper right chondrosternal region, of nearly 10 year duration. The lesion was excised including the removal of portions of the sternum and the II right rib, with the operative diagnosis of a tumor of uncertain nature.

PATHOLOGY

GROSS FEATURES

The gross view showed a firm, non-encapsulated, well-circumscribed, 10 cm sized neoplasm. The cut surface was speckled by necrotic foci.

MICROSCOPIC FINDINGS

Histologically at low magnification a biphasic appearance was the main feature with epithelioid and spindle cell patterns, the latter organized mainly in fascicular arrangements. At higher power an oncocytic change of the cytoplasm of tumor cells was visible in both the epithelioid and spindled areas. A myxo-hyaline chondroid-like matrix was also present with focal pooling of mucin.

IMMUNOHISTOCHEMISTRY

Positive immunostains were those for vimentin, specific and alpha smooth muscle actins, glial-fibrillary acidic protein, and particularly strong was the one for mitochondrial antigens (113-1MoAb). Weak staining for S100 protein. No immunoreactivity for epithelial membrane antigen, cytokeratins, HMB-45, desmin, myoglobin, CD34.

ELECTRONMICROSCOPICAL ANALYSIS

Ultrastructurally the epithelioid and spindle cells were embedded in a proteoglycan-rich extracelllular matrix. Their cytoplasm was filled up with packed mitochondria which appeared enlarged and swollen due to the paraffin retrieval procedure. Desmosomes and arrays of actin-type microfilaments were focally seen. Tracts of basal lamina were also present, and mostly interesting some of both the epithelioid and spindled tumor cells showed extensive cytoplasmic accumulations of intracisternal microtubules.

DIAGNOSIS

Biphasic, spindle and epithelioid cell myoepithelioma of soft tissue, oncocytic, type.

DISCUSSION

Myoepithelioma is a rare tumor composed of spindle, plasmacytoid, epithelioid, and clear cells with immunohistochemical and electron-microscopical features of myoepithelial differentiation (1-2), in almost total absence of ductal epithelial structures. Normal myoepithelial cells exhibit the hybrid immunohistochemical and ultrastructural phenotype of both mesenchymal contractile cells and epithelial cells. Immunohistochemically myoepithelial cells are usually positive for cytokeratins, S100 protein, actins, glial fibrillary acidic protein, and calponin. Ultrastructurally they exhibit a variable content of intermediate filaments of vimentin-type, focal densities resembling smooth muscle dense bodies, pinocytotic vesicles, some desmosomes with tonofibril-like bundles, and foci of basal lamina. Neoplastic myoepithelial cells usually parallel the double morphofunctional

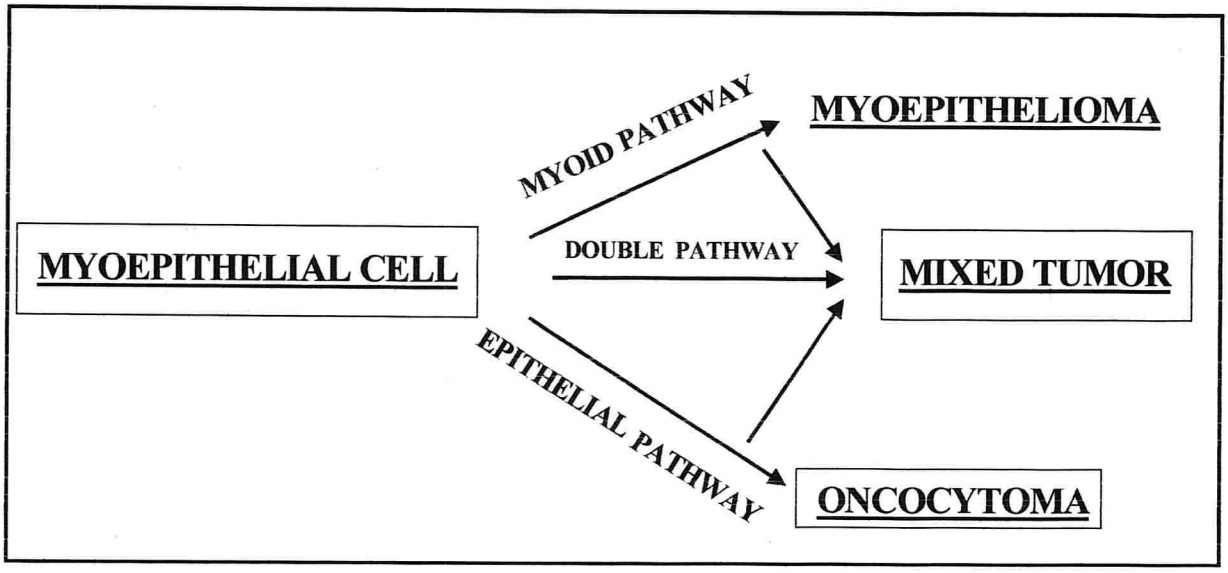
phenotype of normal epithelium, although they often can express either epithelial or myoid unbalanced differentiation. The relatively common sites of occurrence for myoepitheliomas are represented by salivary glands and breast. Genuine soft tissue myoepitheliomas, including both cutaneous and deeply seated examples are extremely rare tumors with scarce reference in literature: they have been only recently recognized with a total 33 cases so far reported, including the present case (case 3 –ref. 11), occurring in the dermis, in the subcutaneous and subfascial planes, in intramuscular or parosteal compartments, and even in retroperitoneum -one case (3-12). Soft tissue myoepitheliomas have been observed in a wide range of age, since childhood to old age. Size is quite variable -from 2-20 cm- and location is represented by extremities, head & neck, and trunk. Most of the cases so far reported have been described microscopically as comprised of spindle cell, even though mixed type (epithelioid and spindle cell), and myxoid type have also been recorded. A pure epithelioid example has even been encountered in the skin (personal unpublished observation). No mention concerning with the existence of an oncocytic variant in the family of mioepytheliomas has been found in the previous literature, except in ref. 3 which actually reports on this very present case. Thus, according to the reporting authors (3-8,11), the differential diagnosis –pending on morphology, size and location- considers a wide range of lesions, benign and malignant, among which tumors of neural lineage (neurilemmoma, neurofibroma, MPNST), smooth muscle cell tumors, soft tissue perineurioma, synovialsarcoma (mainly of the fibrous monophasic type, or even biphasic type as in the present case), fibrosarcoma, myofibrosarcoma, solitary fibrous tumor, spindle cell lipoma, low grade fibromyxoid sarcoma, myxoid liposarcoma, myxoid chondrosarcoma, and even metastatic carcinoma and follicular dendritic cell sarcoma. Also, for peculiar locations, such as the skin and the retroperitoneum, we should include other plausible although exotic possibilities, such as the hyaline cell rich chondroid syringoma (13,14), and the adenomyoepithelioma (15,16) in the former location, and the extra-gastrointestinal stromal tumor (17,18) and extra-pancreatic solido-cystic pseudopapillary tumor in the latter (19). Further, oncocytic myoepitheliomas –such as the present case- may pose diagnostic problems versus all those mesenchymal neoplasms with oncocytic changes (e.g. rhabdomyoma, epithelioid leiomyosarcoma, glomus tumor, paraganglioma, plasmacytoma, Spitz nevus, melanoma, ...), which however still retain the light microscopical, electronmicroscopical and immuno-histochemical features of the cell lineage from which they are derived (20,21).
Finally –although of lesser clinical significance- mixed epitheliomas, spindle and epithelioid cell type must be differentiated from the so-called mixed tumors of soft tissue as well as from the exceptional evenience of a pure oncocytoma of soft tissue, which according to some authors are all congeners (4,11). Myoepithelioma, mixed tumor, and oncocytoma have been proposed as members of a unique rubric of tumors, this implying not only the credit for a central role played by the myoepithelial cell (clearly endowed of the ability to differentiate along both the epithelial and mesenchymal lines) but also the hypotesized common origin for all of them from the myoepithelium of (supposedly) deeply and ectopically located adnexal sweat glands: thus accordingly myoepithelioma would represent one end of the spectrum along the myoid line of differentiation and oncocytoma the other end following the epithelial line, while the mixed tumor is in the middle expressing morphological features of both lineages (Tabl.1).

Ultrastructurally (3,7,11) myoepithelioma of soft tissue is quite similar to the salivary gland counterpart, exhibiting a variable content of intermediate filaments of vimentin-type, focal densities resembling smooth muscle dense bodies, pinocytotic vesicles, some desmosomes

with tonofibril-like bundles, and foci of basal lamina. Most notably, the biphasic epithelioid and spindle cell myoepithelioma herein presented appears unique in that most of the cells, irrespectively of their shape, showed in addition to extensive cytoplasmic collections of mitochondria even numerous intracisternal microtubules. The presence of intracisternal microtubules is an unexplained features. Intracisternal microtubules are commonly found in extraskeletal myxoid chondrosarcomas, malignant melanomas and few other tumorous conditions (24). Their recognition in soft tissue myoepitheliomas further expands the spectrum of tumors possibly exhibiting intracisternal microtubules with this notion being helpful in order to avoid erroneous diagnostic conclusions.
The clinical behaviour of myoepitheliomas (and mixed tumors) is consistent with a low-grade malignancy with 10-20% metastatic rate via lymphatic or hematogenous route (4,6, present case: cf Follow-up).

Follow-up: thirty months after the surgical intervention, the patient manifested lung metastases which were surgically excised and histologicaly found corresponding to the epithelioid tumor cell component (sections kindly provided by dr. G. La Stilla, Anatomic Pathology, University of Bari, Bari).
Aknowledgment: We thank prof. CDM Fletcher, MD (Brigham & Women's Hospital, Boston, MT, USA), and prof. V. Eusebi, MD (University of Bologna, Bologna, Italy) for their assistance in the study of this case.

TABLE 1. DIAGRAM OF PATHOLOGICAL DIFFERENTIATION OF MYOPEPITHELIAL CELL.



REFERENCES

1. Dardick I. Myoepithelioma: definitions and diagnostic criteria. Ultrastruct Pathol 1995;9:335-345.

2. Martinez-Madrigal F and Micheau C. Myoepithelial cells. In: Sternberg SS, ed. Histology for Pathologists. New York, NY: Raven Press, 2nd ed.; 1997:415-417.
3. Burke T, Johnson DE, Ordöñez NG, Mackay B. Myoepithelioma of the retroperitoneum. Ultrastruct Pathol 1995;19:269-274.
4. Kilpatrick SE, Hitchcock MG, Kraus MD, Calonje E, and Fletcher CDM. Mixed tumors and myoepitheliomas of soft tissue. A clinicopathologic study of 19 cases with a unifying concept. Am J Surg Pathol 1997;21:13-22.
5. Fernàndez-Figueras M-T, Puig L,Trias I, Lorenzo J-C, and Navas-Palacios J-J. Benign myoepithelioma of thwe skin. Am J Dermatopathol 1998; 20: 208-212.
6. Michal M, & Miettinen M. Myoepitheliomas of the skin and soft tissues. Report of 12 cases. Virchows Arch 1999;434:393-400.
7. Sasaguri T, Tanimoto A, Arima N, Hamada T, Hashimoto H, Sasaguri Y. Myoepithelioma of soft tissue. Pathol Int 1999;49:571-576.
8. Fletcher CDM, Kutzner HK, Fernàndez Figueras M-T, Conzàles S. Cutaneous myoepithelioma? Am J Deramtopathol 2000; 22:360-361.
9. Bisceglia M, Dimitri L, Scaramuzzi F, Pasquinelli G. Il tumore misto, il mioepitelioma, e l'oncocitoma, delle parti molli: una inusuale categoria di nuove entità tumorali. Pathologica 2000; 92: 475-476.
10. Bisceglia M, Cardone M, Dimitri L, Pasquinelli G, e Cenacchi G. Monotematica II. Seconda parte di "excerpta". Entità rare delle parti molli,di recente delineazione e di possibile riscontro in dermatopatologia: il lipoma condroide, il tumore misto, il mioepitelioma, l'oncocitoma. G Ital Dermatol Venereol 2001; 136 (Suppl. 1 al n. 1): 12-14.
11. Bisceglia M, Cardone M, Fantasia L, Cenacchi G, and Pasquinelli G. Mixed tumors, myoepitheliomas, and oncocytomas of the soft tissues are likely members of the same family. A clinicopathologic and ultrastructural study. Ultrastruct Pathol 2001; 25:399-418.
12. Bisceglia M, Dimitri L, Magro G, Mantovani W, Pasquinelli G. I mioepiteliomi della cute e delle parti molli: studio morfologico,immunoistochimico, e ultrastrutturale di 4 casi. G Ital Dermatol Venereol 2002; 137 (Suppl 2 al n. 1): 115-120.
13. Ferrero JA, Nascimento AG. Hyaline cell-rich chondroid syringoma. A tumor mimicking malignancy. Am J Surg Pathol 1995; 19: 912-917.
14. Bisceglia M, Clemente CR, Vairo M, Cafaro A, Pasquinelli G. Hyaline cell-rich chondroid syringoma. Report of a case with morphological, immunohistochemical, and ultrastructral analysis and review of the literature. Pathologica 1997; 89: 540-545.
15. Wallis NT, Banerje SS, Eyden BP,Armstrong GR. Adenomyoepithelioma of the skin: a case report with immunohistochemical and ultrastructure observations. Histopathology 1997; 31: 364-377.
16. Losi L, Perrone A, Eusebi V. Tumore epi-mioepiteliale (adenomioepitelioma) della vulva: descrizione di un caso. Pathologica 1998; 90; 788-791.
17. Reith JD, Goldblum JR, Lyles RH, Weiss SW. Extragastrointestinal stromal tumors. Mod Pathol 2000;13:577-585.
18. Miettinen M, Lasota J. Gastrointestinal stromal tumors –definition, clinical , histological, immunohistochemica,and molecular genetic features and diffferential diagnosis. Virchows Arch 2001; 438:1-12.
19. <<The 1st "*Arkadi M. Rywlin International Pathology Slide Seminar*" Symposium in Anatomic Pathology>>. June 19-21, 2002. Syllabus: Case 34 (presented by Masaharu Fukunaga).

20. D'Amore EG, & Ninfo V. Tumors of the soft tissue composed of large eosinophilic cells. Sem Diagn Pathol 1999;16:178-189.
21. Suster S. Tumors of the skin composed of large cells with abundant eosinopjhilic cytoplasm. Sem Diagn Pathol 1999;16:162-177.
22. Polk P, Parker KM, and Biggs PJ. Soft tissue oncocytoma. Hum Pathol 1996;27:206-208.
23. Bisceglia M, Dimitri L, Armilotta M, Tardio B. Soft tissue oncocytoma: an additional case report. Pathologica 1998;90:201-202.
24. Erlandson RA. Diagnostic electron microscopy of tumors. New York, NY: Raven Press; 1994:162-163.

QUESTIONS

1. Soft tissue myoepitheliomas are considered benign tumors.
   False or True. _____.
2. Name at least two malignancies in which intracisternal microtubular structures are found.
   Indicate the mane: ....., ...

1. I mioepiteliomi delle parti molli sono ritenuti tumori benigni.
   Falso o Vero. _____.
2. Nominare almeno due tumori maligni in cui si può riscontrare all'esame ultrastrutturale la presenza di strutture microtubulari intracisternali.
   Indicare i nomi: ....., ...

CASE 10 01-25679

Kumarasen Cooper, M.D., Department of Pathology, University of Vermont, Burlington, Vermont, USA

CLINICAL HISTORY

The excised lesion is from a mass on the right foot in a 28-year-old woman. Her history was that of a slowly growing painless mass.

PATHOLOGICAL FINDINGS

The tumor was received as multiple tan-yellow to white firm fibrous soft tissue fragments measuring 5.0 x 3.0 x 1.5 cm. The cut surfaces were tan-yellow and focally gelatinous.

Microscopically, this tumor is characterized by nodules of myxoid tissue surrounded by cellular to hyalinized stroma, variable inflammatory cells and large atypical cells with prominent nucleoli. On low power, the regional variation between myxohyaline areas and cellular granulation tissue-like areas with inflammation is notable. The bizarre atypical cells are interspersed throughout non-myxoid stroma and the paucicellular myxoid tissue. The appearance of the bizarre-shaped nuclei spans the spectrum from ganglion-like cells to Reed-Sternberg-like cells to cells mimicking lipoblasts. The ganglion-like cells have a large, vesicular, irregularly shaped nucleus and a huge nucleolus. The cytoplasm is prominent and spindled forms are also present. The binucleated forms of these cells resemble Reed-Sternberg cells. The multivacuolated lipoblast-like cells are found in the myxoid areas and feature hyperchromatic, enlarged, sometimes indented nuclei in addition to cytoplasmic vacuoles. Occasional scattered multinucleated giant cells are identified. The stroma outside the myxoid nodules alternates between cellular and hyalinized areas. Within the myxoid nodules the cells vary from being spindled to epithelioid with mild to moderate nuclear atypia (except for the bizarre cells). The hyalinized areas are prominent and contain only a few tumor cells with thick-walled vessels. Mitoses, including atypical forms, are present but difficult to find.

The inflammatory cells are most prominent in the non-myxoid cellular areas and complete the histologic picture. The composition is that of lymphocytes, plasma cells, polymorphonuclear leukocytes (especially within the myxoid areas), and eosinophils. Occasionally the intense inflammation may partially obscure the large atypical cells.

DIAGNOSIS

Inflammatory myxohyaline tumor of distal extremities IMHT (Acral myxoinflammatory fibroblastic sarcoma).

In summary, the essential criteria for a diagnosis of IMHT is the location on distal extremities, myxoid nodules scattered about hyalinized to cellular stroma, large bizarre ganglion-like cells or lipoblast-like cells and a mixed inflammatory cell infiltrate.

DISCUSSION

Inflammatory myxohyaline tumor of distal extremities is a neoplasm of low malignant potential. It is characterized by large ganglion-like cells, myxoid nodules and a stroma that varies from being cellular to hyalinized, with associated inflammation.

This tumor was first published in 1998 with the appearance of three simultaneous reports comprising significant numbers of patients. The descriptive labels used by these authors were remarkably similar: "Inflammatory myxohyaline tumor of distal extremities" (Weiss), "Acral myxoinflammatory fibroblastic sarcoma" (Kindblom) and "Inflammatory myxoid tumor of soft parts with bizarre giant cells" (Michal). Although the term "sarcoma" was used by one of the authors, distant metastases have not been demonstrated in the 63 patients with follow-up; and only 1 patient developed regional lymph node metastasis. Nevertheless, two-thirds of patients in one series and one-fifth in the other developed one or more recurrences. Hence, the authors of the AFIP fascicle on soft tissue tumors assign this tumor to the intermediate group of neoplasms, rather than the sarcoma group; hence the label "inflammatory myxohyaline tumor" (IMHT) is preferred.

A total of 75 cases have been reported to date. The age of patients has ranged from childhood to the ninth decade. The majority are in the fifth and sixth decades. These tumors affect the sexes equally. Almost all tumors occurred in the hands/fingers (two-thirds), feet/toes, ankle/wrists, whilst a few involved the arms/lower legs. Significantly, no tumors developed on the trunk, head/neck, or within body cavities. Patients present with a history of a slowly growing painless mass, most often in the subcutaneous tissue. The lesions ranged in size from 1 to 8 cm (median, 3 to 4 cm). They frequently infiltrate synovium, subcutaneous fat and dermis. However, epidermal and bone invasion has not been reported. Clinically these lesions were suspected to be ganglion cysts, tenosynovitis, or giant cell tumors of tendon sheath. A single case report with cytogenetic analysis showed t(1;10)(p22;q24) in addition to the loss of chromosomes 3 and 13.

IMMUNOHISTOCHEMISTRY

Immunohistologic examination of a total of 35 cases from both series demonstrated vimentin positivity in all atypical cells. A variable number of atypical cells was also immunopositive for CD68 and CD34. Weak, focal smooth muscle actin and keratin immunopositivity were also demonstrated in a few cases. The inflammatory infiltrate comprised a mixture of both B and T cells, especially the latter. The S-100, HMB-45 and epithelial membrane antigen were negative in the neoplastic cells.

DIFFERENTIAL DIAGNOSIS

- Tenosynovitis may be considered, especially when the heavy inflammatory infiltrate obscures the large atypical cells and when the tumor growth involves the synovial lining of a tendon sheath. The finding of the bizarre cells should avoid this error.
- Inflammatory myofibroblastic tumor (IMT) (inflammatory pseudotumor) enters the differential diagnosis due to the presence of a spindle cell component and the inflammation in IMHT. However, the bizarre cells of IMHT are not present in IMT. Further, IMT practically never occurs in the distal extremities.

- Ganglion cysts and juxta-articular myxomas do not contain the large atypical cells found in IMHT. Further, juxta-articular myxomas involve larger joints and do not exhibit the increased focal cellularity of IMHT.
- Proliferative fasciitis is also characterized by ganglion-like cells. However, the ganglion-like cells of IMHT are much more atypical than those of proliferative fasciitis, which also lacks marked inflammation.
- Neural tumors with enlarged nuclei and myxoid stroma are S-100 positive; whilst the cells in IMHT are negative.
- Myxoid liposarcoma enters the differential diagnosis as a result of the lipoblast-like cells. However, myxoid liposarcoma does not feature the large atypical nuclei and never occurs in the distal extremities. The characteristic plexiform vasculature and signet ring lipoblasts are not features of IMHT.
- Pleomorphic liposarcoma is rare in acral locations and the large multivacuolated cells in IMHT are not true lipoblasts.
- Extraskeletal myxoid chondrosarcoma is not characterized by the marked inflammation, fibrosis and cellular atypia of IMHT.
- Atypical lipomatous tumors contain atypical cells but primarily comprise adult fat.
- Epithelioid sarcoma can have significant inflammation, contain scattered atypical polygonal and spindle-shaped cells and often originates in the superficial tissue of the distal extremities. However, the majority of the cells in epithelioid sarcoma are round cells with eosinophilic cytoplasm. When large atypical cells are present, they are usually cytokeratin positive, whilst similar cells are cytokeratin negative in IMHT. Even when positive in the latter, it is focal and weak.
- Myxofibrosarcoma (myxoid MFH) on purely histologic evidence is fraught with difficulty. However, the clinical presentation on the distal extremity, paucity of mitotic figures, ganglion -like or Reed-Sternberg-like cells, and marked inflammation are useful features to recognize IMHT.

TREATMENT AND PROGNOSIS:

The recurrence rate in the follow-up of the patients in the two published series of IMHT was 22 percent and 67 percent. Some of the recurrences were multiple and aggressive enough to warrant amputation. In one patient, the recurrence extended up the arm. Recurrences may also occur more than a decade after the initial excision. The efficacy of post-operative radiation is difficult to assess given the prolonged course of many of these tumors. Although spread to a regional lymph node has been documented in one case, distant metastases has not been reported to date. Hence, IMHT appears to have a recurring potential but its metastatic capacity (to date) is very low. In the Kindblom series, 31 percent persisted with disease, 64 percent were alive without disease, and 5 percent were dead of other causes. No patient had died of IMHT at the last follow-up.

REFERENCES

1. Kempson RL, Fletcher CDM, Evans HL, Hendrickson MR, Sibley RK: Inflammatory myxohyaline tumor of distal extremities (acral myxoinflammatory fibroblastic sarcomas). In: Atlas of Tumor Pathology: Tumors of the Soft Tissues, AFIP Press, 2001, pg 86-89.
2. Lambert I, Debiec-Rychter M, Guelinkx P, et al: Acral myxoinflammatory fibroblastic sarcoma with unique clonal chromosomal changes. Virchows Arch 2001; 438:509-512.

3. Meis-Kindblom J and Kindblom L-G: Acral myxoinflammatory fibroblastic sarcoma. A low-grade tumor of the hands and feet. Am J Surg Pathol 1998; 22:911-924.
4. Michal M: Inflammatory myxoid tumor of the soft parts with bizarre giant cells. Pathol Res Pract 1998; 194:529-533.
5. Montgomery EX, Devaney KO, Giordano TJ et al: Inflammatory myxohyaline tumor of distal extremities with virocyte or Reed-Sternberg-like cells: a distinctive lesion with features simulating inflammatory conditions, Hodgkin's disease, and various sarcomas. Mod Pathol 1998; 11:384-391.

QUESTIONS

1. What is the biological behavior of these neoplasms?
   - **A.** Low grade.
   - **B.** Intermediate.
   - **C.** High grade.
   - **D.** No metastatic potential.
   - **E.** No capability of recurrence.
2. What is the current recommended management of these tumors?
   - **A.** Chemotherapy.
   - **B.** Radiotherapy.
   - **C.** Complete local resection only.
   - **D.** Wait and see.
   - **E.** Complete local resection with follow-up.

***Mark the appropriate letter.**

1. Quale è il comportamento biologico di queste neoplasie?
   - **A.** Basso grado.
   - **B.** Intermedio.
   - **C.** Alto grado.
   - **D.** Nessun potenziale metastatico.
   - **E.** Nessuna capacità di recidiva.
2. Quale è il trattamento attualmente raccomandato per questi tumori?
   - **A.** Chemioterapia.
   - **B.** Radioterapia.
   - **C.** Asportazione locale completa.
   - **D.** Nessun trattamento, ma seguirne l'evoluzione.
   - **E.** Asportazione completa locale con "follow-up".

***Contrassegnare la lettera giusta.**

CASE 11. 3357-90

Janez Lamovec, M.D., Institute of Oncology, Ljubljana, Slovenia.

HISTORY

A 23-year-old patient presented with a recurrent lesion of the left femoral head. This was the third recurrence in two years; previously, tumor was twice curetted at another institution and diagnosed as chondroblastoma. This time the segmental resection of the femur was performed.

PATHOLOGY

Grossly, the specimen was represented by a 10 cm long segment of proximal left femur with some surrounding soft tissue. On cut surface, the tissue of the head and neck of the femur appeared spongy, with unevenly sized hemorrhagic cavities. There were also some sclerotic areas seen, and focally, white-bluish small nodules of cartilage like tissue were noticed. In the region of the great trochanter, the fracture fissure through corticalis was seen..

Microscopically, this is a highly hemorrhagic lesion, with blood in numerous vascular spaces, in spaces without clear endothelium as well as in interstitium. The most characteristic feature of the tumor are clear cells, of uniform appearance, with distinctly demarcated cell borders and centrally placed round to oval nucleus and usually distinct nucleolus. In many cells, the cytoplasm is more eosinophilic. Mitoses are rare. Cells are arranged in vague small lobules separated by delicate stroma. In many areas, they are separated by pale homogeneous basophilic matrix and are more cartilaginous in appearance. Few foci of conventional low to intermediate grade chondrosarcoma are also present. In most areas, benign osteoclastic giant cells are scattered among neoplastic cells. In almost all areas of the tumor, irregular bone and also osteoid trabeculae are dispersed in the neoplastic tissue, some trabeculae are clearly rimmed by osteoblasts. In some foci, tumor simulates the appearance of osteoblastoma or osteosarcoma, elsewhere the tumor mimics aneurismal bone cyst or chondroblastoma. Focally, tumor tissue infiltrates and surrounds preexistent bony trabeculae.

PAS staining, with and without diastase digestion showed no glycogen granules (fixation in formalin, decalcification).

Immunohistochemically, the tumor cells were strongly vimentin and S-100 positive, and negative for CKMNF116, EMA, GFAP, CD99, bcl-2; osteoclastic cells were positive for CD68.

DIAGNOSIS

Clear cell chondrosarcoma.

FOLLOW UP

The patient is alive and well 11 years after segmental resection of the femur and hip replacement.

COMMENT

Clear cell chondrosarcoma (CCC) of the bone was first described as specific entity in 1976 by Unni et al. (1). In 1984, the same group of authors published 47 cases of this tumor and thus further delineated its morphological and clinical characteristics (2). This is a rare tumor, representing around 2% of all chondrosarcoma cases (3).

Clinically, most patients are in the third or fourth decade, they are more commonly of male sex, and present with local pain, often of long duration.

On X-ray, the lesion is constantly found in proximal and rarely in distal ends of long bones, involving both epiphysis and metaphysis. Generally, the lesions are radiolucent but may contain areas of calcification within them, usually fluffy in appearance. Margins of the lesion toward adjacent bone are often sclerotic. The bone is usually expanded. The cortex of bone may be thinned out and may show pathologic fracture.

Grossly, the lesions are soft, or more solid and gritty; rarely cystic. The cartilaginous nature of the tumor is not always obvious grossly.

Microscopically, the hallmark of the tumor are clear cells, polygonal or round in shape, relatively uniform, with well defined borders and centrally placed oval nuclei of generally uniform size and shape with distinct nucleoli. The cytoplasm often show perinuclear or peripheral eosinophilic condensation. Mitoses are rare. Lobular arrangement of tumor cells is common. Osteoclast-like giant cells are frequently admixed. The characteristic and almost constant feature of the tumor is the presence of heterogeneous osteoid/bone trabeculae within the tumor which appear to be reactive/metaplastic. The lesion may be well vascularized. The associated changes due to previous biopsy/ies may modify the characteristic morphological presentation. In some cases, aneurismal bone cyst-like, osteosarcoma or osteoblastoma-like, chondroblastoma-like and giant-cell tumor-like changes may dominate in the histological appearance of the tumor. In a substantial number of cases, areas of convential chondrosarcoma are also part of the tumor (2-4). Immunohistochemically, tumor cells show strong positivity for S-100 protein (4).

In the differential diagnosis, other chondroid tumors should be considered, such as conventional chondrosarcoma and chondroblastoma. The former may show vacuolization of tumor cells producing clear multivacuolated cells, individual or in small clusters, separated by cartilaginous matrix and easily distinguished from polygonal cohesive, almost epithelioid clear cells in CCC. In addition, osteoclast-like giant cells and reactive bone trabeculae are practically never found in conventional chondrosarcoma (2-4). Chondroblastoma is always a diagnosis to be considered, also witnessed by our case, particularly in those cases of the latter tumor that appear beyond the second or third decade of age. Chondroblastoma shows lobularity, calcifications, vascularity and giant cells just like CCC but cells of chondroblastoma are not clear, nuclei are not round but indented, and no reactive osteoid or bone trabeculae are present. Chicken wire type calcifications, characteristically present in chondroblastoma are not observed in CCC (3-6).

In addition, CCC must be differentiated from other bone tumors which may, regularly or occasionally contain clear cells, such as metastatic clear carcinoma (e.g., from kidney, breast, et.), from other primary bone tumors such as clear cell osteosarcoma , Ewing's sarcoma/PNET, and exceptionally, in bones most unusual for CCC, from chordoma and ameloblastoma. In addition, clear cell sarcoma of soft tissues may secondarily involve bone, either by direct spread or by metastatic involvement. Of other benign lesions, osteoblastoma may also be a potential possibility. In cases of classical clinical, roentgenological and histological appearance of clear cell chondrosarcoma, none of the latter lesions present serious differential diagnostic problems (4). In less typical cases, particularly in those with pronounced secondary changes, the diagnosis may be more difficult. Immunohistochemical reactions are helpful in certain circumstances – particularly the reactions for epithelial markers, S-100 protein, and neuroendocrine markers.

The possible relationship of CCC with chondroblastoma remains undetermined; some authors consider it to be a malignant form of chondroblastoma (7). The chondroblastic nature of the tumor appears to be generally accepted (8-9), with some opposing views (10).

The most important aspect of this variant of chondrosarcoma is its low grade biological behaviour although the tumor may occasionally metastasize. Metastases may appear many years following surgery and the need for long-term follow-up has been emphasized (11). Local recurrence usually follows inadequate surgical procedure, e.g. curetting; segmental

resection of the affected bone is a treatment of choice. In rare cases, the tumor may "dedifferentiate", producing a component of high grade sarcoma with a tendency to develop lethal metastatic disease (12).

REFERENCES

1. Unni KK, Dahlin DC, Beabout JW, et al. Chondrosarcoma: clear cell variant; a report of sixteen cases. J Joint Bone Surg (A) 1976; 58: 676-683.
2. Bjornsson J, Unni KK, Dahlin DC, et al. Clear cell chondrosarcoma of bone. Observation in 47 cases. Am J Surg Pathol 1987; 8: 223-230.
3. Present D, Bacchini P, Pignatti, et al. Clear cell chondrosarcoma. A report of 8 cases. Skel Radiol 1991; 20: 187-191.
4. Swanson PE. Clear cell tumors of bone. Sem Diagn Pathol 1997; 14: 281-291.
5. Kurt AM, Unni KK, Franklin H, et al. Chondroblastoma of bone. Hum Pathol 1989; 20: 965-976.
6. Edel G, Ueda Y, Nakanishi J, et al. Chondroblastoma of bone. A clinical, radiological, light and immunohistochemical study. Virchows Arch A Patho Anat 1992; 421: 355-366.
7. Yamanishi H, Isu K, Ubayama Y, et al. Clear cell chondrosarcoma. A report of two cases and review of the literature. Acta Pathol Jpn 1986; 36: 1577-1585.
8. Aigner T, Dertinger S, Belke J, et al. Chondrocytic cell differentiation in clear cell chondrosarcoma. Hum Pathol 1996; 27: 1301-1305.
9. Masui F, Ushigome S, Fujii K. Clear cell chondrosarcoma: a pathological and immunohistochemical study. Histopathology 1999; 34: 447-452.
10. Bosse A, Ueda Y, Wuisman P, et al. Histogenesis of clear cell chondrosarcoma. An immunohistochemical study with osteonectin, a non-collagenous structure protein. J Cancer Res Clin Oncol 1991; 117: 43-49.
11. Bagley L, Kneeland JB, Dalinka MK, et al. Unusual behavior of clear cell chondrosarcoma. Skel Radiol 1993; 22: 279-282.
12. Kalil RK, Inwards CY, Unni KK, et al. Dedifferentiatiate clear cell chondrosarcoma. Am J Surg Patholl 2000; 24: 1079-1086.

QUESTIONS

1. Clear cell chondrosarcoma represents ?% of all chondrosarcoma cases:
   - **A.** 0.1%
   - **B.** 2%
   - **C.** 10%
   - **D.** 25%
2. Which differential diagnosis in clear cell chondrosarcoma is the least probable:
   - **A.** chondroblastoma
   - **B.** conventional chondrosarcoma
   - **C.** fibrous dysplasia
   - **D.** osteoblastoma
   - **E.** osteosarcoma

***Mark the appropriate letter.**

1. Il condrosarcoma a cellule chiare rappresenta in che percentuale incide fra tutti i condrosarcomi?
   - **A.** 0.1%
   - **B.** 2%
   - **C.** 10%
   - **D.** 25%
2. Quale entità –tra quelle di sotto elencate- meno probabilmente entra in diagnosi differenziale:
   - **A.** Condroblastoma.
   - **B.** Condrosarcoma convenzionale.
   - **C.** Displasia fibrosa.
   - **D.** Osteblastoma.
   - **E.** Osteosarcoma.

***Contrassegnare la lettera giusta.**

CASE 12. BP95-336

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CLINICAL HISTORY

A 16-year-old Japanese girl presented with a 6-month history of pain in the distal and inside part of the left thigh. Physical examination, computed tomography scan, and ultrasound examination showed a well-circumscribed mass measured 3.5 cm in the greatest diameter with the left muscularis vastus medialis. Excision was undertaken. She was alive with no evidence of disease three years after excision.

PATHOLOGY

MACROSCOPICAL FEATURES

The excised tumor, measuring 3.5 X 2.5X 2.0 cm, was well circumscribed and appeared as a white mass with a fibrous to myxoid cut surface:

HISTOLOGY

The tumor was not encapsulated. Relatively hypercellular areas with collagenous stroma altered with less cellular areas in which tumor cells were interspersed with myxoid stroma. Vessels were relatively prominent in fibrous areas. The tumor showed predominantly haphazard, whorled or short fascicular arrangements. The cells were uniform and had a benign appearance; they were spindle shaped and had fusiform or ovoid, vesicular nuclei with fine chromatin, inconspicuous nucleoli and scant indistinct cytoplasm. The tumor infiltrated into adjacent skeletal muscles. Neither cellular atypia nor mitotic figures were observed. There was neither necrosis nor hemorrhage.

Special stains: vimentin (++), alpha-smooth muscle actin (+), desmin (+), HHF35 (-), S-100 protein (-), CD34 (-), CAM5.2 (-), EMA (-).

DIAGNOSIS

Low-grade fibromyxoid sarcoma (LGFMS).

DISCUSSION

The first two cases of LGFMS were described by Evans (1) in 1987, and a further case report (2) appeared in 1990. LFGMS occur equally in men and women and typically involve young adults. LGFMS typically presents as s slowly growing, deeply seated mass of the proximal extremities or trunk. This neoplasm is characterized by the combination of a remarkably bland morphology and a tendency to metastasize. The classic histological features are poor to moderate cellularity, a proliferation of bland-appearing spindle tumor cells with ill-defined palely eosinophilic cytoplasm, and alternating fibrous and myxoid areas with a whorled pattern of the tumor cells. Mitotic figures are few. No defined differentiation is observed by light microscopy. Immunohistochemical and ultrastructural studies suggest a fibroblastic nature. In a recent study by Folpe (3), approximately 10% of cases showed areas with increased cellularity and nuclear atypia, similar to that seen in usual-type fibrosarcomas of intermediate grade. This does not appear to be an adverse prognostic factor when present in small areas. Among 27 reported cases, 15 showed local recurrence and 9 had lung metastases.

The interval to lung metastasis ranged from zero (metastases at presentation) to 45 years (median, 5 years) (1,2,4-7).
In 1997 Lane et al. (8) described the hyalinizing spindle cell tumor with giant rosettes (HSCT) as a distinctive tumor closely resembling low-grade fibromyxoid sarcoma. Subsequent studies (3,9) have strongly suggested that HSCT is, in fact, simply a LGFMS with uniquely prominent collagen rosette formation. Fibrohyalinized nodules were observed in the present case, however, no of them showed typical rosettes-like figures.
Differential diagnosis includes low-grade myxofibrosarcoma, desmoid fibromatosis, and perineurioma. Low-grade myxofibrosarcoma differs from LGFMS in that it has generally occurred in older adults, is always predominantly myxoid and composed of fusiform cells with hyperchromatic atypical nuclei, and does not metastasize. Desmoid fibromatosis is poorly defined and shows a greater degree of cellularity, prominent fascicular proliferation, and more interstitial collagen fibers. Perineuriomas have many of the histologic features of LGFMS, including a whorling pattern and relatively bland cells. Positive immunostain for EMA in perineurioma helps to distinguish it from LGFMS.
It is very important to keep in mind that there is a potential of LGFMS for late metastases, and a close and long clinical follow-up is essential.

REFERENCES

1. Evans HL. Low-grade fibromyxoid sarcoma. A report of two metastasizing neoplasms having a deceptively benign appearance. Am J Clin Pathol 1987; 88:615-9.
2. Devaney DM, Dervan P, O'Neill S, Carney D, Leader M. Low-grade fibromyxoid sarcoma. Histopathology 1990; 17:463-79.
3. Folpe AL, Lane KL, Paull G, Weiss SW. Low-grade fibromyxoid sarcoma and hyalinizing spindle cell tumor with giant rosettes: a clinicopathologic study of 73 cases supporting their identity and assessing the impact of high-grade areas. Am J Surg Pathol 2000; 24:1353-6.
4. Goodlad JR, Mentzel T, Fletcher CDM. Low grade fibromyxoid sarcoma: clinicopathological analysis of eleven new cases in support of a distinct entity. Histopathology 1995; 26:229-37.
5. Fukunaga M, Ushigome S, Fukunaga N. Low-grade fibromyxoid sarcoma. Virchows Arch 1996; 429:301-3.
6. Ugai K, Kizaki T, Morimoto K, Sashikata T. A case of low-grade fibromyxoid sarcoma of the thigh. Pathol Int 1994; 444:793-9.
7. Evans HL. Low-grade fibromyxoid sarcoma. A report of 12 cases. Am J Surg Pathol 1993; 17:595-600.
8. Lane KL, Shannon RI, Weiss SW. Hyalinizing spindle cell tumor with giant rosettes: a distinctive tumor closely resembling low-grade fibromyxoid sarcoma. Am J Surg Pathol 1997; 21:1481-8.
9. Nielsen GP, Selig MK, O'Connell JX, Keel SB, Dickersin GR, Rosenberg AE. Hyalinizing spindle cell tumor with giant rosettes: a report of three cases with ultrastructural analysis. Am J Surg Pathol 1999; 23:1227-32.

QUESTIONS

1. What is the differential diagnosis of LGFMS?
   List some entities: ....., ..., ...
2. Does LGGMS have potential for late metastasis? **Yes or No.** ____.

1. Quale è la diagnosi differenziale del "sarcoma fibromixoide di basso grado?"
   Elenca alcune condizioni: ....., ..., ...
2. Il "sarcoma fibromixoide di basso grado" ha capacità di dare metastasi tardive?
   Si o No. ____.

CASE 13. 7753-01

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CLINICAL PRESENTATION AND PATHOLOGIC DESCRIPTION

The patient is a 66 yr old recent immigrant from the ex-Soviet Union in general good health who presented with complaints of progressive swelling and pain in the left knee. X ray examinations of the area (including CT) showed a destructive lesion of the left distal femur focally penetrating through the cortex, while MRI showed definite extension into the soft tissues . Other systemic radiological examinations failed to reveal additional lesions elsewhere save for a liver finding judged to be hemangioma. Needle biopsies of the lesion yielded two cores of friable bone measuring about 2x0.5 cm. Histologic examination revealed bone fragments, some appearing non-viable, mixed with a spindle cell proliferation also containing what were perceived to be mixed reactive inflammatory cells (including leukocytes and some plasma cells), and fibrous tissue. The spindle cells in part looked bland but with areas of atypia, some were thin and arrayed in parallel bundles as in a desmoplastic reaction, while others were plumper with a vague patterning. Some of the fragments which looked fibrotic may actually be necrotic. I didn't notice mitotic activity (even in retrospect).

My impression was of a chronic sclerosing osteomyelitis. I discussed the matter with the orthopedist also mentioning the atypia (which I thought was possibly degenerative or reactive). He appeared satisfied with the diagnosis and based on my written diagnosis proceeded with a definitive procedure. This yielded more abundant larger tissue fragments, many of which on macroscopic examination showed fat tissue at the periphery, greyish tissue in the middle to a width of between 0.5-1.0 cm, and softer paler tissue in the center. The slide distributed to you is from this second procedure, and as you can see, there is a florid expansile spindle cell proliferation which seems to bulge into the peripheral fat tissue but not clearly invasive into it (in the sense that the tumor seemed well demarcated without individual tumor cells infiltrating into the fat). There is a florid plasma cell proliferation at the periphery as if to wall off the tumor from the non-involved soft tissues. There is more noticeable atypia and mitotic activity in this material, with obvious confluence of the proliferating cells which may have been equivocal in the original biopsies (at least to me). The inner portion shows degeneration/necrosis with fibrosis and also with numerous (at least in foci) neutrophils. To me at the time this seemed to me to be a sort of (pseudo)zonation phenomenon (though without maturation of tissue) which led me astray. The material was sent to Dr Chris Fletcher of Brigham Hospital in Boston who set me straight and made the diagnosis of intermediate grade leiomyosarcoma (strong extensive SMA positivity, desmin negative). Following receipt of the diagnosis the patient was referred to a dedicated orthopedic oncology unit at another hospital where above the knee amputation was performed. Examination revealed a 10 cm mass occupying the distal femur and penetrating into the knee joint, with additional nodular lesions outside it. Slides from that specimen kindly provided me by the pathologist at that institution show an obvious spindle cell malignancy with little inflammatory infiltrate. The cells are a bit plumper and more eosinophilic than in the previous material. However immunohistochemical stains performed at the other institution for desmin, actin, and keratin were negative, and the diagnosis made there was undifferentiated sarcoma.

DIAGNOSIS
Leiomyosarcoma of femur.

DISCUSSION:
Leiomyosarcoma of bone is infrequently diagnosed. Based on the treatment given in the standard references I have available (1 and 2), fibrosarcoma or malignant fibrous histiocytoma would be more common diagnoses in this situation. Leiomyosarcoma is described amongst "miscellaneous mesenchymal lesions" in the first source and in the second, is mentioned in passing. It should be pointed out that the location of the tumor in this case would be usual for any of these entities, none of which show a particular age predilection. In the literature there are multiple case reports and a few series reported, the largest one based on the material at Memorial Hospital (3). That series consists of 33 cases collected over a period of 19 years (less than two per year, though they rejected patients with more than minor soft tissue involvement and any patient with a previous leiomyosarcoma elsewhere and women who had undergone hysterectomies for leiomyomata or for unknown reasons; since the latter proscriptions may affect many women it is possible that there is a more sizeable incidence of the disease as diagnosed pathologically than these numbers would indicate). There was one 13 yr old patient, the remainder were aged 21 to 77 yrs. (one peak in the third decade, most of the others between 40-70 yrs.). These tumors showed a proclivity for the area around the knee but otherwise were not concentrated in any particular age group. Most were high grade; interestingly there was not much necrosis in the sections examined which according to the authors reflects the tendency of pathologists to avoid sampling areas which look necrotic. I should add that the material given me from the subsequent amputation also showed little necrosis which may reflect the luxury afforded the pathologist of determining her sampling while the sampling in the material I dealt with was performed by the surgeon. All cases in this study were SMA positive, while 50% were desmin positive (not all leiomyosarcomas are desmin positive).

I asked Prof Fletcher how many cases of primary bone leiomyosarcoma he sees. He replied about 3 patients seen in his hospital and an equal number of cases sent in consultation per year. He feels that this tumor is probably more common than is usually granted and that the frequency of its being diagnosed may reflect traditions and prejudices established before the era of immunohistochemistry.

While this lesion is uncommon enough (or unusual as you wish) to justify its inclusion in this seminar, my particular interest in discussing this is as a learning experience which I wish to share with my colleagues. Obviously we are all aware of the pitfalls of small biopsies which may be taken from non-optimal areas. While osteomyelitis is considered rare in older people, I should point out that while I don't see many biopsies of bone lesions in my general hospital practice, many of them in the end turn out to be infectious or inflammatory/reactive in nature, including those in elderly people and notwithstanding the high clinical and radiological suspicion and subsequent hysterics of the clinician upon receiving the diagnosis. Thus I was not put off as such from making the diagnosis I originally made which statistically could be supported by the idiosyncracies of my own personal experience. In retropect I should have been more cautious concerning the atypia especially in light of the fact that the inflammation as it was was not overwhelming and diffuse but rather focal in distribution. I didn't have immunohistochemistry performed on the biopsy, but since the subsequent tumor cells

stained only for actin, it is questionable if such a procedure would have been helpful in of itself in differentiating granulation tissue/reactive myofibroblasts from true tumor.

I would be happy and in fact grateful for any comments or criticisms regarding the handling of the case.

REFERENCES

1. Fechner R.E. and Mills S.E. Tumors of the bones and joints. Atlas of Tumor Pathology. Fascicle 8, Third Series. Armed Forces Institute of Pathology Washington D.C., 1993: p. 205-206
2. Unni K.K. Dahlin's Bone Tumors, fifth edition. Philadelphia: Lippincott-Raven, 1996: p 201, 205-6
3. Antonescu C.R. et al. Primary leiomyosarcoma of bone. Am J Surg Pathol. 1997; 21: p. 1281-1294

QUESTIONS

1. Positive desmin reactivity is essential and necessary for a malignant tumor to be diagnosed as leiomyosarcoma. **True or false.** _____.
2. A lesion in the same location with the same radiological picture in a young adult may be a sarcoma. **True or false.** _____.

1. La positività immunoistochimica è necessaria ed essenziale per la diagnosi di un leiomiosarcoma. **Vero o Falso.** _____.
2. Una lesione nella stessa sede con lo stesso quadro radiologico in un giovane adulto può essere un sarcoma. **Vero o Falso.** _____.