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Detection of *Paracoccidioides brasiliensis* by PCR in biopsies from patients with paracoccidioidomycosis: correlation with the histopathological pattern

Dimostrazione di *Paracoccidioides brasiliensis* con PCR in biopsie di pazienti con paracoccidioidomicosi: correlazioni con le lesioni istologiche

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Key words

*Paracoccidioides brasiliensis* • Paracoccidioidomycosis • PCR • Mycosis • Granuloma

Summary

Paracoccidioidomycosis, a systemic mycosis, is rarely diagnosed in its initial phase and can remain latent for up to 40 years. Although PCR is sensitive for the identification of *Paracoccidioides brasiliensis* (Pb) in different samples, no study using paraffin-embedded human tissue has been published. The size of the amplicon, the fixation method and the time of the storage may affect the reaction. Recently the more sensitive Primer-Extension-Preamplification (PEP)-Nested-PCR has been used for amplification of small samples. Our aims were to detect Pb in paraffin embedded biopsies using (PEP)-Nested-PCR and to correlate the data with histopathological parameters. Analyses were carried out in 107 biopsies from tegument, lymph node, lung and tongue. The fungal DNA was detected in 29.9% of the biopsies by (PEP)-nested-PCR against 5% of Nested-PCR. The positivity correlated with numbers of fungi and fungal viable cells, and there was no correlation with the granuloma pattern.

Introduction

Paracoccidioidomycosis (PCM) is a deep granulomatous mycosis caused by *Paracoccidioides brasiliensis* (Pb). It is a serious, at times fatal, disease of difficult early diagnosis, which may remain latent for up to 40 years. The granulomatous inflammatory response to the fungus can be classified as compact or loose according to the greater or lesser extent of organization of the histiocytic and epithelioid cellular response. A definitive diagnosis can be made by ancillary testing such as culture, direct examination, serology or histo-

Acknowledgements

The work was supported by a FAPESP grant (n° 01/07563-3). The Authors would like to thanks: i) Dr. Celso R. Silva; Dr. Luis A. Veronez; Dr. Margarida M.F.S. Moraes; Dr. Ivo Ricci; Dr. Marco A. Avó; Dr. Lucimar R. Avó; Dr. Jose C. Prates; Dr. Antonio P. Pereira; Dr. Francisco C. Quevedo; Dr. Odashiro Maçanori, Dr. Fernando T. Mota and Dr. Roque Galhardo Filho for providing the paraffin blocks; ii) Dr. Zoilo Pires de Camargo for providing the *H. capsulatum* DNA sample, iii) Naiara Corrêa Nogueira de Souza, Mr Antonio Carlos de Souza and Mr Joaquim Soares de Almeida for technical assistance, iv) Ms Maria C. Aparecida do Nascimento for secretarial assistance.

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pathology. Since 1995, new more specific techniques based on the polymerase chain reaction (PCR) have been used and reported to be faster, efficient and able to detect differences between strains. The high sensitivity and specificity of PCR for detection of Pb was then documented in serum, blood, fresh lung tissue, fixed biopsy from experimentally infected animals and soil samples.

Recently, another technique known as primer extension pre-amplification (PEP) has been developed. The technique has been showed to be useful in samples containing little material since produces DNA copies from one single cell after multiple rounds of extension. It is believed that 78% of the genomic sequence from a single haploid cell can be at least 30 times copied. Although PCR is sensitive and specific for the identification of Pb in various types of samples, so far no study testing the technique in paraffin-embedded tissue in human biopsies has been published.

The size of the fragment to be amplified, the fixation technique and the time of the storage of the material are known to affect the efficacy of the method when applied in paraffin-embedded samples. Furthermore, factors related to the characteristics of the paracoccidioidic granuloma, such as the pattern of the inflammation and the quantity and viability of the fungal cells, may influence the sensitivity of the reaction.

Material and methods

We analyzed 107 biopsies of different tissues from patients with PCM (92 of tegument, 6 of lymph nodes, 8 of the lungs and 2 of tongues) fixed in 10% formalin and embedded in paraffin. The samples were obtained from several Laboratories of Anatomic Pathology in Brazil. The biopsies were undertaken from 1983 to 2000 and previously diagnosed by histology as paracoccidioidomycosis, which was confirmed by two independent pathologists.

Histopathology

The blocks were cut into 3 µm-thick sections, which were stained with hematoxylin-eosin (HE) and Gomori-Grocott. Histopathological examination was carried out in order to determine the presence of the following parameters:

i) Compact granuloma characterized by epithelioid tubercles, presence of multinucleated giant cells of the Langhans type and of a lympho-mononuclear cellular halo; Loose granuloma characterized by ill-defined epithelioid cellular aggregates intermingled with an exudative, necrotizing inflammatory reaction;

ii) Fungal viability, as identified by HE and Gomori-Grocott staining according to the following criteria: Viable fungal cells – intact basophilic protoplasm with preserved yeast-like shape; Inviable fungal cells – loss of protoplasm basophilia, and vacuolated and/or collapsed cells. Viable and inviable fungal cells counting were performed on five high power fields (HPF) in a random manner, and the results were reported as the average number of fungal cells per HPF;

iii) Fungal burden: total fungal cells counting were performed randomly on 5 HPF and results were reported as the average number of fungal cells per HPF.

DNA extraction

DNA was extracted using the DEXPAT® kit (TAKARA, Otsu, Japan), which is designed for paraffin-embedded samples. Sequential 5 to 10 µm thick sections were obtained from the paraffin blocks and placed in tubes containing 1.0 ml of the extraction solution, boiled at 90°C for 10 min and then centrifuged for 15 min at 13,000 rpm at 4°C. The supernatants were stored at -20°C until the time for PCR.

PCR amplification

Primer-Extension Pre-amplification (PEP) – PCR

After DNA extraction, to increase the gene expression of the samples, PEP reaction was performed according to the protocol using a 15 bp degenerated primer. The PCR mix consisted of 5 µl of the DNA extraction, 20 mM Tris-HCl buffer, pH 8.0, containing 50 mM KCl, 2 mM MgCl₂, 1 mM deoxynucleoside triphosphate and Taq DNA polymerase (5 U/µl). The primers were added to a final concentration of 1 ng per 50 µl of reaction (all the products were from Invitrogen®). The reactions were performed in a GeneAmp® – PCR System 9700 – thermocycler for fifty cycles of 94°C (3 min), 94°C (1 min), 55°C (4 min), 72°C (30 sec) and then 72°C (5 min).

PCR

A sequence from human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used in order to check the presence of amplifiable DNA according to the protocol. The outer primers were gap 1 (5'-GAC AAC AGG TCA GGT GGA GGA-3') and gap 2 (5'-ATG CCA GTG AGC TTC CCG-3') and which amplified fragments of 325pb. The PCR mix consisted of 10 µl of DNA extraction, 20 mM Tris-HCl buffer, pH 8.0, containing 50 mM KCl, 2 mM MgCl₂, 1 mM deoxynucleoside triphosphate and Taq DNA polymerase (5 U/µl), The primers were added to a final concentration of 1 ng per 50 µl of reaction. The reactions for the outer primers were performed in a thermocycler for thirty-five cycles of 94°C (5 min), 94°C (30 s), 56°C (30 s), 72°C (45 s) and then 72°C (5 min). The second PCR protocol, with the inner primer, was identical except that it was used 2 µl of the 1st reaction product and 40 cycles were carried out (all products from Invitrogen®).

Nested-PCR

The procedure was performed according to the protocol. We also used the set of primers based on gp43 suggested by the Authors: primers. Outer: 1-5’ AAC TAG AAT ATC TCA CTC CCA GTC C 3’ 2-5’ TGT AGA CAT GCT TGC ATG TCT TGG
Detection of *P. brasiliensis* by PCR and correlation with histopathology

G-3’ which are complementary to the position 846 to 870 and 1200 to 1176 of the GenBank sequence, respectively, defining a 355-nucleotide amplicon.

Inner: III-5’ GAT CGC CAT CCA TAC TCT CGC AAT TGC G 3’ which are complementary to the nucleotide positions 953 to 978 and 1148 to 1124 respectively, and amplify fragments of 196 bp.

1st Reaction: the PCR mix consisted 1 µl of the PEP product, 20 mM Tris-HCl buffer, pH 8.0, containing 50 mM KCl, 2 mM MgCl₂, 1 mM deoxynucleoside triphosphate and Taq DNA polymerase (5 U/µl). The primers were added to a final concentration of 1 ng per 50 µl of reaction. The reaction for the outer primers set was performed in the thermocycler for thirty-five cycles of 95°C (5 min), 95°C (30 sec), 50°C (30 sec), 72°C (1 min) and then 72°C (5 min). The 2nd reaction, for the inner primers set, consisted with 1 µl of the product of the 1st reaction and the mix concentrations was the same described above in a total volume of 25 µl thermocycled for 30 times of 95°C (5 min), 95°C (30 sec), 72°C (1 min) and then 72°C (5 min) (all products from Invitrogen®).

Negative control: DNA of *Histoplasma capsulatum* was used in all reactions. Either DNA samples extracted from culture or paraffin material were analyzed.

Positive control: DNA of Pb, extracted from culture. Gel: the PCR products (10 µl) were separated by electrophoresis on ethidium bromide-stained 2% agarose gels and the band intensities were analyzed using the Kodak Digital Science – EDAS 120 system. The molecular weight standard used was the 100 bp DNA Ladder (Invitrogen®).

Statistical analysis: data were analyzed statistically by the Chi-square test for qualitative variables and by the non-parametric Mann-Whitney and Kolmogorov-Smirnov tests or the Shapiro-Wilk test for quantitative variables.

### Results

Out of the 107 tested samples by PCR, 29.0% (32 samples) were positive and 71.0% (75 samples) were negative by PEP-Nested-PCR (Fig. 1). With only Nested PCR, the results were positive in less than 5% of the samples.

### Discussion

The 30% positivity of PCR for the detection of *P. brasiliensis* in the biopsies may have been due to factors such as:

i) the paraffin blocks were collected from several laboratories with different procedures used for fixation, paraffin embedding and storage of the material, events that may have favored DNA degradation in some samples;
ii) the use of primers of regions of the gp43 gene that have proved to be polymorphic, a fact that may have interfered with the perfect annealing of the primers used.

The correlation between PCR positivity and fungal burden was an expected data since the higher the numbers of fungal viable cells, the higher the probability of some intact DNA.

As described in the literature, the histopathological diagnosis of PCM may be difficult in the pauci-parasitic lesions as well as in lesions with the presence of the diminute forms of the agent which may be mistaken by *Histoplasma capsulatum* (Hc), fungus with similar morphology. DNA of *Hc* was then used as a negative control in our testing.

Currently some molecular studies based on Pb DNA sequences have been published. For the molecular PCR diagnosis of PCM, the primers used were based initially on the gp43 and lately from other sources, such as 5.8S rDNA and ITS, which showed higher sensitivity. As our samples were formalin fixed and paraffin embedded, we decided to use gp43 primers, since primers based on ITS region amplify bigger fragments, and our DNA extraction kit did not recommend the use of primers amplifying fragments above 400 bp.

The interference of formalin and tissue paraffin embedding with the DNA integrity for later use in molecular techniques has been the subject of several investigations, which have demonstrated that both formalin and paraffin damage DNA. In addition, the deparaffinization of the block, which requires organic solvent such as xylene, in high temperature (60 °C), may also damage DNA. It is of interest that the DNA extraction kit employed in the present study did not include the use of xylene and deparaffinization steps. On the other hand, the absence or a low concentration of the desired product may be due to the presence of other materials that compete with the DNA amplification, such as patient’s DNA, excess protein in the sample and presence of microorganism contaminants in the block. These factors may form nonspecific background bands that compete with the DNA to be amplified.

Since archival paraffin blocks are crucial for retrospective studies employing modern molecular pathology methodology further studies on PCR standardization in the diagnosis of deep mycoses are warranted.

### Conclusion

The sensitivity of PEP-Nested-PCR for detection of Pb in PCM biopsies fixed in 10% formalin and embedded in paraffin is superior to Nested-PCR. There was a correlation between fungal quantity and viability, and PEP-Nested-PCR positivity. No correlation was detected between PEP-Nested-PCR positivity and the pattern of the granulomatous inflammation.

### References

Detection of P. brasiliensis by PCR and correlation with histopathology


Panel di ausilio diagnostico nella patologia proliferativa uroteliale: studio degli Antigeni Citocherratina 20, p53, CD44 e Ki-67

A useful panel in proliferative urothelial lesions: an analysis of Cytokeratin 20, p53, CD44 and Ki-67 Antigens

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Introduzione

La finalità del patologo nell’interpretazione di un preparato di biopsia vescicale è quella di dirimere la biologia della lesione e, in quelle neoplastiche, di chiarirne l’aggressività. Infatti in vescica, accanto a lesioni produttive, ve anche aggettanti, talora di tipo reattivo-metaflogistico, si annoverano rare forme neoplastiche sicuramente benigne, ma, soprattutto, esistono numerose lesioni il cui comportamento biologico non è sempre prevedibile in base all’aspetto morfologico. Dopo multiple recidive, infatti, si può assistere ad un improvviso aumento dell’aggressività della neoplasia che supera i confini anatomici dell’organo e/o va incontro a disseminazione metastatica. Meno frequenti sono quelle lesioni il cui comportamento è francamente maligno sin dalla loro prima presentazione. Queste ultime sono le più insidiose perché spesso il sintomo cardine della storia clinica, l’ematuria, è tardivo e si presenta quando la neoplasia è già profondamente estesa nello spessore della parete. L’obiettivo che ci si è posti è quello di descrivere i quadri istopatologici delle lesioni clinicamente sospette, morfogeneticamente correlate a processi non neoplastici e di proporre un algoritmo diagnostico con metodiche immunoistochimiche di supporto nella diagnosi differenziale, spesso insidiosa, tra le proliferazioni reattive e quelle neoplastiche in situ. I marcatori usati, infine, sono stati valutati sui carcinomi uroteliali di basso ed alto grado, anche infiltranti.

Un’importante caratteristica dell’urotelio è la sua capacità di modificare il suo aspetto morfologico in relazione a stimoli locali. Sono state descritte numerose

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variazioni morfologiche reattive della mucosa vescicale che, ricorrendo in soggetti adulti per il resto del tutto normali, ed essendo molto comuni, non sono considerate espressione di condizioni patologiche.

McKenney JK, Desai S, Cohen C, et al., sulla base di precedenti esperienze che avevano valutato la p53 e la citocheratina 20 (CK20) nelle neoplasie uroteliali, hanno associato a questi anticorpi, la molecola di adesione CD44 (isoforma standard) nel tentativo di elaborare un panel utile nella distinzione tra CIS e quelle forme di atipia reattiva il cui aspetto morfologico si presta ad incertezze interpretative. Nel loro lavoro gli A.A. sottolineano che è importante utilizzare i tre anticorpi perché non in tutti i casi di CIS vi è chiara espressione immunooistochimica per questi marker. Infatti i risultati da loro raccolti mostrano diversi pattern specifici: l’urotelio normale è positivo alla CK20 solo nello strato superficiale delle cellule ad ombrello, con la colorazione per il CD44 limitata agli strati basale e parabasale, mentre la colorazione nucleare per p53 varia da assente a focale; l’urotelio con atipia reattiva mostra reattività per il CD44 in tutti gli strati, soprattutto nei casi con atipia marcata. CK20 e p53 hanno invece gli stessi pattern di reattività dell’urotelio normale; il CIS mostrerebbe una forte positività citoplasmatica per la CK20 estesa a tutte le cellule proliferanti e diffusa positività nucleare per p53 in tutto lo spessore uroteliale. Se residua un sottile strato di cellule basali normali, esso sarà ancora positivo al CD44, altrimenti non compare nelle cellule neoplastiche. Tali risultati sono stati verificati correlando il pattern immunooistochimico a casi di certa diagnosi morfologica controllati con studi retrospettivi correlati dal follow-up dei pazienti.

Sulla base di questo lavoro si è voluta controllare la riproducibilità di questi risultati su materiale routinario, allo scopo di inserire queste metodiche nell’algoritmo diagnostico delle lesioni cistoscopicamente ed istologicamente sospette.

Si è valutata, inoltre la distribuzione e l’intensità dell’attività proliferativa dell’urotelio, con Ki 67 (MIB 1), nei quadri reattivi, nel CIS e nelle neoplasie uroteliali papillari diasso ed alto grado.

### Materiali e metodi

Presso l’Unità Operativa Complessa di Anatomia Patologica dell’Ospedale dei Pellegrini di Napoli dal luglio 2003 al luglio 2006 sono stati esaminati 698 campioni bioppticiei vescicali, da cui sono stati selezionati 81 campioni di materiale biptico vescicale comprensenti 50 casi di neoplasia uroteliale, 5 controlli normali e 26 lesioni uroteliali “mimic”.

I campioni bioppticiei sono stati fissati in formalina ed inclusi in paraffina. La colorazione immunooistochimica è stata condotta con il coloratore automatico Ventana Benchmark, utilizzando il metodo immunoperossidassico indiretto streptavidina-biotina; gli anticorpi monoclinali anti-CK20 (clone Ks20.8 Ventana), p53 (clone Bp53-11 Ventana) e MIB 1 (clone K-2 Ventana) sono stati prediluiti. L’Ab anti-CD44v6 (clone VFF-7 Labvision-Neomarker) è stato adoperato alla diluizione di 1:40. L’immunoreattività è stata valutata contando il numero di cellule positive in 10 campi microscopici ad alto ingrandimento (×40), equivalenti a 2 mm² di tessuto.

### Risultati

Le colorazioni immunoistocheamiche dell’urotelio normale mostrano positività per CK20 a livello del citoplasma delle cellule ad ombrello in 5/5 (100%) dei casi esaminati, e marcano solo rari elementi superficiali residui eventualmente presenti nell’urotelio reattivo in 24/26 dei casi (92,30%). Nel carcinoma intraepiteliale, invece, tutte le cellule atipiche risultano CK20 positive nei 50 casi osservati (100%). Il p53 è assente o blandamente e solo focalmente positivo nell’urotelio normale (100%) e reattivo (84,6%-22/26 casi), mentre diventa diffusamente positivo, con intensità variabile nei nuclei del carcinoma di basso grado (80% 20/25 casi) e alto grado (100% 25/25 casi). Il CD44 è risultato marcato la membrana citoplasmatica delle cellule basali nell’urotelio normale nel 100% dei casi e quella di quasi tutti gli strati cellulari nell’iperplasia reattiva in 25/26 casi (96,15%). La colorazione scompare nelle cellule del carcinoma di basso grado in 23/25 casi (92%) e nel carcinoma di alto grado in 24/25 casi (96%). Il Ki67 ri-

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### Tabella 1. Quadro sinottico dei risultati ottenuti.

<table>
<thead>
<tr>
<th></th>
<th>Ki67 basale</th>
<th>Ki67 diffuso</th>
<th>P53 focale</th>
<th>P53 diffuso</th>
<th>CK20</th>
<th>CD44</th>
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<tr>
<td>Urotelio normale (5)</td>
<td>5</td>
<td>0</td>
<td>5/debole</td>
<td>0</td>
<td>5 (superficiale)</td>
<td>5 (basale)</td>
</tr>
<tr>
<td>Urotelio reattivo (26)</td>
<td>24</td>
<td>2</td>
<td>22/debole</td>
<td>4</td>
<td>24 (superficiale)</td>
<td>25 (diffuso)</td>
</tr>
<tr>
<td>Carcinomi di basso grado (25)</td>
<td>3</td>
<td>23</td>
<td>5</td>
<td>20/intenso</td>
<td>25 (diffuso)</td>
<td>2</td>
</tr>
<tr>
<td>Carcinomi di alto grado (25)</td>
<td>0</td>
<td>25</td>
<td>0</td>
<td>25/intenso</td>
<td>25 (diffuso)</td>
<td>1</td>
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Presentato come poster (dati preliminari) al Congresso Nazionale SIAPEC svoltosi a Venezia nell’ottobre 2006.
sulta positivo con una distribuzione basale nell’urotelio normale e nelle iperplasie reattive rispettivamente nel 100% e 92,30% (24/26) dei casi. Nel CIS, invece, sono presenti nuclei positivi in tutti gli strati dell’urotelio con una concentrazione nettamente superiore. È interessante notare che le cellule reattive residue nei carcinomi in situ pagetoidi risultano caratteristicamente positive per CD44 in contrasto con gli elementi neoplastici negativi. Di converso la marcatura con cK20 evidenzia le cellule neoplastiche, frammiste agli elementi uroteliali reattivi. Le neoplasie uroteliali di basso grado hanno mostrato: positività diffusa e marcata per CK20, positività debole o di media intensità per la p53 (80% – 22/26 casi) e positività di alcuni nuclei, diffusi però in tutti gli strati per il Ki67 (92% – 23/25 casi). Si è notato, infatti, che più che il numero dei nuclei marcati, rispetto all’urotelio reattivo, variava la loro disposizione che risultava “anarchica”, con una caratteristica maggiore concentrazione nelle cellule degli strati superficiali. Nelle neoplasie uroteliali di alto grado, infine, p53 e Ki67 marcavano diffusamente i nuclei di quasi tutte le cellule neoplastiche (nel 100% dei casi) che risultavano, inoltre, diffusamente positive per CK20 (100%). La ricerca per CD44, così come nei casi di CIS, risultava negativa in quasi tutti i carcinomi uroteliali papillari (23/25 basso grado; 24/25 alto grado).

**Discussione**

Questo panel ha mostrato una potenziale utilità in aggiunta alla morfologia in diverse situazioni diagnostiche: in casi nei quali il patologo propende fortemente per la diagnosi di CIS ma nutre ancora dei dubbi sulla risposta definitiva; in casi nei quali si pone una diagnosi di CIS senza storia nota di pregressa lesione papillare (CIS de novo o primario); nella conferma di presentazioni morfologicamente inusuali di CIS come la cancerizzazione di urotelio normale. Le neoplasie uroteliali sia piatte che papillari, indipendentemente dall’infiltrazione, hanno mostrato elementi neoplastici positivi nel 99% per CK20 e p53 e sempre negativi per CD44.
Nessuna neoplasia ha mostrato contemporanea negatività o bassa colorazione per gli anticorpi CK20 e p53.
La distribuzione e l’intensità del Ki67 è risultata la seguente:
- urotelio normale: rare cellule basali;
- urotelio reattivo: sparse cellule basali, con rare cellule parabasali;
- carcinoma in situ: diffusa marcatura, presente anche negli strati superficiali.
I carcinomi papillari di basso grado hanno mostrato tutti un numero di cellule marcate inferiore a quello dei carcinomi di alto grado.

Bibliografia

Case report

Paraganglioma: report of a rare case with ovarian involvement

Paraganglioma: descrizione di un caso con coinvolgimento ovarico

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Key words
Ovary • Paraganglioma • Neuroendocrine Tumor

Parole chiave
Ovaio • Paraganglioma • Tumore Neuroendocrino

Summary
We report a well-documented case of paraganglioma involving right ovary, which was initially misdiagnosed as a Sertoli-Leydig cell tumor and recurred one year later. The right ovarian tumor measured 105x90x60 mm and was associated to a sub-diaphragmatic tumor measuring 80x60x35 mm, a peritoneal and a preureteral nodules measuring 10 mm either. Microscopically, tumor cells were arranged in trabeculae and cords separated by a delicate stroma. Their cytoplasm was abundant granular and eosinophilic. Their nuclei were enlarged and regular in size with coarse chromatine and a large nucleolus. The tumor expressed neuroendocrine markers (chromogranin, synaptophysin) epithelial membrane antigen and focally cytokeratin 7 and e-cadherin. Pathological ovarian paraganglioma diagnosis could be difficult but one should be aware of its bona fide existence. The clinical course is favourable in most of the cases.

Riassunto
Viene descritto un caso di paraganglioma coinvolgente l’ovaio destro, che inizialmente era stato diagnosticato come tumore a cellule di Sertoli-Leydig e che, ad un anno di distanza dal primo intervento, ha recidivato. La massa neoplastica ovarica destra aveva dimensioni di 105x90x60 mm ed era associata ad una neoplasia sottodiafragmatica di 80x60x35 mm e a due noduli, uno peritoneale e l’altro preureterale, entrambi di 10 mm di diametro massimo. Microscopicamente le cellule neoplastiche, caratterizzate da nuclei voluminosi e di forma regolare con cromatina granulare e nucleolo evidente, presentavano crescita in trabecole e cordoni separati da un esile tessuto stromale. Le cellule neoplastiche sono risultate positive per marcatori neuroendocrini (Cromogranina, Sinaptofisina) ed EMA, con espressione focale di Cito-cheratina 7 e Camerina E. La diagnosi istologica di paraganglioma ovario può essere difficoltosa, e pertanto bisogna quantomeno essere consapevoli della possibile esistenza di tale entità. Il decorso clinico è, nella maggior parte dei casi, favorevole.

Introduction

Extra-adrenal paragangliomas are very uncommon tumors arising from neuroectodermal-derived tissue. While these tumors often occur in the superior or inferior para-aortic regions, there are reports of their presence in virtually every organ. Ovary paragangliomas are extremely rare tumor. Only 5 cases have been described in the literature. They are categorized along with other miscellaneous tumors and tumor-like conditions in the recent World Health Organisation classification of tumors of the breast and female genital organs.

Since this lesion is relatively rare, we report a well-documented case of paraganglioma involving ovary.

Case report

A 29-year-old woman was admitted for recurrence of pelvic mass revealed one year ago for menstrual irregularity. The mass was developed at the surface of the ovary and the initial pathologic report advocated Sertoli-Leydig cell tumor. Physical examination revealed a mass overleading the douglas in rectal examination. Serum endocrine testing (testosterone, 17 hydroxy-progesterone and delta 4 androstenedione) and CA125 level

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were normal. Other routine laboratory studies were unremarkable.

The abdominal and pelvic Computed Tomographic Scan demonstrated two 70 and 45 mm pelvic solid masses compressing the uterus, the bladder and the right urethra. Masses were separated from adrenal glands and the kidney. No tumor was seen in either adrenal gland. A 68 mm well-circumscribed mass was also noted below left diaphragm. Masses were moderately enhanced after injecting intravenous product.

At laparotomy, the right ovary was largely replaced by a 8 cm solid tumor. A 7 cm mass was noted below the left diaphragm. A 2 cm peritoneal and preureteral firm nodules which were removed.

This patient underwent an hysterectomy with bilateral salpingo-oophorectomy, a rectal resection, an omentectomy and an appendicectomy.

On gross examination, the right ovary measured 105/90/60 mm and the sub-diaphragmatic mass measured 80/60/35 mm. A 10 mm peritoneal and preureteral nodules were associated. On sectioning, they had a multilobulated white/yellow cut surface.

After fixation with formalin, multiple specimens of the tumor were routinely processed for paraffin embedding. Five-micrometer-thick sections were stained with hae-matoxylin and eosin.

Histological examination revealed a similar feature for right ovary, sub-diaphragmatic masses and for peritoneal and preureteral nodules.

Tumor cells were arranged in trabeculae and cords separated by an abundant capillary network. Tumor cells had abundant granular eosinophilic cytoplasm and regular ovoid nuclei containing large nucleoli. Cellular atypia, mitosis, necrosis and vascular invasion were absent. Immunohistochemical analysis was performed by means of the peroxidase-antiperoxidase method. The neoplastic cells were strongly immunoreactive for neuroendocrine markers (chromogranin, synaptophysin) and for epithelial membrane antigen (EMA). Cytokeratin 7 (CK 7) and E-cadherin were focally positive. Stains for
carcinoembryonic antigen (ACE), CK 5/6, vimentin, HBME1, calretinin and inhibin were negative.

No gross or histologic abnormality was identified in the left ovary, the fallopian tubes, the uterus, the rectum, the omentectomy and the appendicectomy. The postoperative course was unremarkable and the patient remains well and has no clinical evidence of recurrence 12 months after the treatment.

Discussion

Ovary paragangliomas are extremely uncommon lesions. The first case was reported in 1971 by Fawcett and Kimbell1, and only 5 cases have subsequently been described in the literature. In addition, two unpublished cases have been described 2. In one case, a particular form was reported consisting in gangliocytic paraganglioma arising from mature cystic teratoma of the ovary 3. Gangliocytic paraganglioma is a distinctive triphasic tumor consisting of epithelioid endocrine cells, spindled schwann-like cells and ganglion cell-like elements 2.

In our case, we consider that ovary could be the primary site which exhibited a malignant behavior, and diaphragm mass, peritoneal and preureteral nodules are secondary sites, since it was the site of the initial tumor and the maximum tumor volume was observed in the ovary. Also against metastasis is the lack, to our knowledge, of a single documented case of paraganglioma metastatic to the ovary.

Age ranges from 15 to 68 years (mean 43.6 years). In three cases, there was a history of hypertension which is secondary to secretion by the tumor of adrenalin and noradrenalin.

Tumor was unilateral in 4 cases and bilateral in only one case. Gross examination revealed a well-circumscribed, solid, tan or brown cut surface tumor measuring from 4 to 22 cm in diameter. Morphologically, these lesions show a characteristic alveolar or nesting (zellballen) pattern. These nests are separated by thin-walled fibrovascular septa. Tumor cells have abundant, granular and acidophilic or clear cytoplasm. Sustentacular cells are positive with inhibin and calretinin in most cases 6,7. In our case, neuroendocrine markers were positive and tumor cells were negative for both inhibine and calretinin. Cytokeratin expression has been initially used to distinguish between paragangliomas, which are negative and carcinoids and neuroendocrine carcinomas, which are positive 8. However, paragangliomas of cauda equina, and 2 of 18 paragangliomas of various sites (orbit, organ of Zuckerkandl, carotid body and vagus nerve) have been found to show intensive, diffuse immunoreactivity for anticytokeratin antibodies (Cam 5.2 and AE1/3) 9. In our case, we noticed a focal expression of CK 7.

Ovary paraganglioma is usually benign 2. The distinction between benign and malignant paraganglioma may be difficult on a purely morphological basis. Some Authors suggested that the presence of sustentacular cells seems to correlate with a benign outcome and paraganglioma that metastasize, like in our case, are more likely to lack sustentacular cells 10.

The histogenesis of ovarian paraganglioma is unknown. Some Authors suggested an origin from extra-adrenal paraganglia in the region of the ovary 11 or unidirectional differentiation within a teratoma 1.

Conclusion

Ovarian paraganglioma is an extremely rare primary neoplasm of unknown aetiology. Only 5 cases have been described in the literature. This tumor must be distinguished from other ovarian tumors containing oxyphilic cells. Further studies of this intriguing neoplasm are necessary to document its full pathologic spectrum.

References


Desmoplastic small round cell tumor of the abdomen
Desmoplastic small round cell tumor of the abdomen.
Report of two cases

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Key words
Desmoplastic small round cell tumor

Summary
Desmoplastic small round cell tumor (DSRCT) is a rare clinico-pathological entity individualized in 1989. Its etiopathogenesis is still unknown, and diagnosis can be achieved only by immunohistochemistry and cytogenetic studies. The objective of this work is to report two new cases of DSRCT and to review the literature to clarify its epidemiological, clinical and pathological aspects.

Introduction
The desmoplastic small round cell tumor (DSRCT) is a highly aggressive tumor recently described by Gerald and Rosai \textsuperscript{1}. This distinctive clinicopathologic entity is characterized by proliferation of small, poorly differentiated cells, separated by prominent desmoplastic stroma. Neoplastic cells exhibit multilineage differentiation, as evidenced by immunohistochemistry or histochemistry, and a specific karyotype abnormality \textsuperscript{2-4}. The tumour shows predilection for young males and predominant intra-abdominal location.

We report herein two new cases of DSRCT and present their epidemiologic and clinicopathologic findings.

Case report n. 1
The patient was an 18-year-old woman who presented with abdominal pain, increased abdominal girth and weight loss. Physical examination showed decreased breath sounds with percussive dullness in the right hemithorax and massive ascites. At laparotomy, ascites and diffuse peritoneal implants were found. A biopsy of the implants was performed. Routine histological sections were prepared from formalin-fixed, paraffin-embedded specimens. Tissue sections were stained with hematoxylin-eosin. They showed a neoplastic proliferation made of sheets of cohesive, small, round, ovoid or

Fig. 1. Sheets of cohesive, small, round, ovoid cells within a hypocellular, collagenous stroma (HE x400).
spindle cells lying within a hypocellular, desmoplastic, collagenous stroma (Fig. 1). Neoplastic cells were round and had oval or elongated hyperchromatic nuclei with clumped chromatin and inconspicuous nucleoli. The cytoplasm was eosinophilic and ill-defined. Mitotic figures were numerous. Immunohistochemical analysis was performed using the avidin-biotin complex technique and antibodies raised against desmin, actin, NSE, vimentin and cytokeratin (Dako). Tumour cells were strongly and diffusely positive for desmin (Fig. 2), actin, NSE, vimentin and, focally, for cytokeratin. The patient died 2 months later.

Case report n. 2

A 25-year-old man was admitted to the hospital because of an eight-month history of discomfort, constipation and weight loss. His past medical history was non-significant. Clinical examination revealed the presence of a painless and firm hypogastric mass. Abdominal ultrasound examination and a computed tomography scan confirmed the presence of a large intraperitoneal mass measuring 12x6x4 cm and showed the occurrence of multiple implants in the peritoneum and of a nodule in the liver. Involvement of the left aortic lymph nodes was also seen. A surgical resection of the large mass was performed. Routine histologic sections were prepared from formalin-fixed, paraffin-embedded blocks. Tissue sections were stained with hematoxylin-eosin and showed a neoplastic proliferation made of sheets and nests of small, round-to-ovoid cells. The stroma was dense and desmoplastic (Fig. 3). Tumour cells exhibited hyperchromatic nuclei with inconspicuous nucleoli and ill defined, eosinophilic cytoplasm. Mitoses were numerous. Immunohistochemical analysis was performed using the avidin-biotin complex technique and antibodies raised against desmin, actin (Fig. 4), NSE, vimentin and cytokeratine (Dako). Tumour cells were strongly and diffusely positive for desmin, actin, NSE and vimentin and, focally, for cytokeratin. A diagnosis of DSRCT was rendered. The patient was treated with six cycles of the following chemotherapy protocol: adriamycin/endoxan for three months and vepeside/cisplatin for four months. The abdominal implants and the nodule of the liver did not significantly decrease in size and multiple bone metastases appeared. A second course of chemotherapy associated with analgetic treatment was instituted. The patient died 18 months after the first hospital admission.

Discussion

DSRCT is a highly malignant mesenchymal neoplasm that frequently arises in the peritoneal cavity of young male patients. Other primary sites have been reported and include the paratesticular region, the pleural serosa, the posterior cranial fossa, soft tissues and bones, the ovary and the parotid gland. Patients’ ages at diagno-
sis have ranged from 3 to 54 years with a median age of 21 years. A male-female ratio of 3:1 is reported in the literature. Clinical manifestations of abdominal DSRCT are usually non-specific, abdominal pain being the most common complaint, followed by weight loss, presence of umbilical hernia, ascites, increased abdominal girth, constipation, hepatomegaly and splenomegaly.

The most characteristic feature of DSRCT at cross-sectional imaging is the presence of single or multiple peritoneal soft-tissue masses, with no apparent organ of origin, located mainly within the omentum or the mesentery, or adjacent to the bladder. Areas of central low attenuation on CT scans appear to correspond to focal haemorrhage within the tumour at gross pathologic analysis. Ascites and hepatic metastases are additional features. CA125 and NSE are frequently raised in the sera of patients with intra-abdominal DSRCT, but cannot be used to reliably monitor the course of the disease.

In recent years, it has become clear that DSRCT constitutes a distinctive neoplastic entity with typical histologic, immunohistochemical and cytogenetic features. On gross examination, DSRCT presents as a solid, firm and multitubulated mass with grey-white cut surface, ranging in size from 1 to 17 cm (mean diameter: 10 cm). Its gross appearance can vary from small, friable omental and peritoneal implants to large, firm omental or mesenteric tumor masses. On histologic examination, DSRCT is composed of solid sheets, large nests, small clumps or cords of cohesive, round, ovoid or spindle cells lying in a hypocellular, desmoplastic collagenous stroma. Neoplastic cells are of small or medium size and show round, oval or elongated hyperchromatic nuclei, clumped chromat, inconspicuous nucleoli and ill defined, lightly eosinophilic cytoplasm. Several mitotic figures are found. Unusual cellular arrangements include the formation of tubules and gland-like or rosette-like structures. Necrosis, at times accompanied by dystrophic calcification, can be seen inside large cellular clusters. The differential diagnosis of DRSCT is usually with Ewing’s sarcoma/primitive neuroectodermal tumor (ES/PNET), embryonal or alveolar rhabdomyosarcoma, Wilms’ tumor and neuroblastoma. When histologic features are not typical, a variety of other neoplastic conditions, such as mesothelioma, rhabdoid tumor, metastatic adenocarcinoma sarcomatoid carcinoma and some spindle cell sarcomas, could enter into the differential diagnosis. DRSCT has a peculiar immunophenotypic pattern characterized by positivity for cytokeratin, EMA, vimentin, desmin and often NSE. Recent cytogenetic studies have identified a recurrent and specific chromosomal translocation: t(11;22)(p13;q12). This translocation results in the fusion of the EWS gene (Wilm’s sarcoma gene) with the WT1 gene (Wilm’s tumor suppressor gene), giving rise to a chimeric RNA transcript composed of the first seven exons of EWS joined to the last three exons of WT1, which can be detected by reverse transcriptase PCR techniques and is considered specific of this tumour. The treatment of DSRCT is a tough challenge consisting of surgery and combination chemotherapy. The majority of patients eventually develop chemotherapy-refractory disease and terminal progression of disease. Consequently, survival remains very disappointing.

References

Desmoplastic small round cell tumor of the abdomen
Primitive neuroectodermal tumor of the kidney with vena caval tumor thrombus: diagnosis and management

Tumore neuroectodermico primitivo del rene con trombosi neoplastica della vena cava: diagnosi e trattamento

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Key words
Primitive neuroectodermal tumor • Kidney • Immunohistochemistry • CD99

Summary
Primitive neuroectodermal tumors (PNET) of the kidney are rare and highly aggressive malignancies. We report a case of 28-year-old male with PNET of the kidney with inferior vena caval thrombus. Immunohistochemistry revealed strong positivity for CD99 and weak positivity for vimentin. Neuron-specific enolase (NSE), chromogranin and cytokeratin were negative. Patient underwent nephrectomy and six cycles of polychemotherapy the patient was in partial remission. He underwent two further cycles of high dose chemotherapy and died 9 months after diagnosis due to liver metastases. The diagnosis of renal PNET must be considered in young patients with renal neoplasm, particularly those with advanced disease at presentation. Achieving exact diagnosis has important clinical consequences.

Introduction
Primitive peripheral neuroectodermal tumor (PNET) is an uncommon malignant neural tumor arising outside the central nervous system. It is composed of small round blue cells that occur predominantly in older children and adolescents. The kidney is rarely involved 1. Only a small number of Authors used the full potential of additional diagnostic procedures, such as immunohistochemistry and genetic testing. Herein, we report on a 28-year-old male patient with a small, round cell renal tumor diagnosed as PNET. We summarize the importance of using currently available diagnostic techniques to render an exact diagnosis and management of PNET.

Case report
A 28-year-old man presented with gross hematuria and right back pain. He had significant loss of weight and appetite during the preceding few months. On physical examination a right renal mass was noted, it was firm in consistency. Computerized tomography of the abdomen revealed an irregular heterogeneous mass measuring 17 x 12 cm completely replacing the right renal parenchyma with extension into the renal vein and vena cava without evidence of metastatic disease. The patient underwent right radical nephrectomy and level II vena caval thrombectomy. On gross examination the tumor measured 15 cm in diameter and occupied the upper pole. It was grey-yellow with multiple areas of haemorrhage and necrosis. The tumor was solid with invasion outside the kidney.

Corrispondenza
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Histologically, the tumor comprised almost uniform population of small round cells. Fine granular changes were found in the cytoplasm, but PAS reaction was negative. Nuclei were round or oval, chromatin was fine and nucleoli were not prominent (Fig. 1). Mitotic figures were found at a high frequency. Extensive areas of necrosis were also identified. The cells were arranged into lobules separated by fibrous vascular septa but perivascular pseudo-rosettes and Homer – Wright-type rosettes were absent. No epithelial structure or heterogeneous mesenchymal elements were found. Immunohistochemistry was performed for cytokeratin, vimentin, CLA, smooth muscle actin, desmin, NSE, CD99, and chromogranin using heat-induced epitope retrieval and the Dako Envision system. There was strong membranous positivity for CD 99 (Fig. 2), occasional cells were vimentin positive. Neuron-specific enolase (NSE), CLA, chromogranin and cytokeratin were negative. The diagnosis of renal PNET was established and six cycles of polychemotherapy (ifosfamide, vincristine, cyclophosphamide, doxorubicin) applied. Thereafter, the patient was in partial remission. Two cycles of high

<table>
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<th>Vimentin</th>
<th>Keratin</th>
<th>Desmin/Actin</th>
<th>LCA</th>
<th>NSE</th>
<th>CD99</th>
<th>S-100</th>
<th>Chromogranin</th>
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<td>-/ +</td>
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<td>–</td>
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<td>+/–</td>
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<td>+</td>
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<td>+/-</td>
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Fig. 1. Monomorphic blue small cell proliferation (HE x400).

Fig. 2. Positive membranous immunotaining for CD 99.
dose chemotherapy with paraplatin and etoposide were administered. Due to liver metastases the patient died 9 months after diagnosis from liver metastasis.

Discussion

Primitive peripheral neuroectodermal tumors include a group of small round cell malignancies of ubiquitous location. There are defined by the presence of a balanced chromosomal translocation (11; 22) (q24; q12) or a (21; 22) rearrangement that always involves the EWS gene at 22q12. The majority of case have presented in the soft tissues and less frequently in the skeletal system, the involvement of the kidney is exceedingly rare. These tumors typically manifest in children and young adults, with a slight male predominance (male:female ~ 1.5:1). Neither clinical evaluation nor radiological methods allow distinguishing PNET from renal cell carcinoma, the diagnosis of renal PNET must be considered in young patients with advanced renal neoplasm at presentation.

The tumor cells are remarkably uniform, possessing a single round hyperchromatic nucleus, an extraordinarily high nuclear to cytoplasmic ratio, and a thin rim of cytoplasmin, sometimes containing small vacuoles indicative of intracytoplasmic glycogen. Nucleoli are generally inconspicuous to absent. Variable numbers of rosettes may be observed, especially in the more differentiated PNET. Mitotic activity tends to be variable and mitoses may be surprisingly sparse. Spontaneous tumor necrosis is frequently present, sometimes leaving residual viable-appearing tumor cells forming collar around blood vessels. The pathological differential diagnosis of malignant small round cell tumors of the kidney includes monophasic Wilms’ tumors (WT), small cell carcinoma, lymphoma, clear cell sarcoma, monophasic synovial sarcoma, neuroblastoma and rhabdomyosarcoma. The diagnosis of PNET is facilitated by immunohistochemical techniques. Antibodies against the MIC2 gene product, such as CD99 may be of diagnosticc help because they stain tumors of the PNET/Ewing’s family. However, B-lymphoblastic type malignant lymphoma, rhabdomyosarcoma and neuroblastoma are positive for MIC2. It is essential to use a panel of antibody including cytokeratin, vimentin, CLA, EMA, desmine, smooth muscle actin (AML), protein S100, neuron-specific enolase (NSE), CD99, and CD34. So the positivity of the CD99 and the negativity of the other antibodies are in favour of the diagnosis PNET. The most PNET express the protein FLI1; but the immunodetection is not absolutely specific of these tumors because it is expressed in benign and malignant vascular tumors and in less constant way, in the other tumors such as carcinomas with Merkel’s cells, melanoma, synovial sarcoma); the utility of this antibody in the diagnosis ES/PNET seems so limited. Jimenez et al. found that 7 of 9 Wilms tumors stained positively for WT1, whereas none of 8 renal PNETs showed positive results. These Authors subsequently proposed that a battery of immunostains to include WT1, CD99, and FLI-1 might reliably distinguish renal ES/PNET from Wilms tumor. Immunohistochemical finding are summarized in Table I. Using reverse transcriptase-polymerase chain reaction (PCR), is an additional tool in the diagnosis. PCR can demonstrate the EWS-FLI1 chimeric gene, a highly specific molecular marker. The optimal method to treat PNET has not been established; however, previous clinical observations show PNET to exhibit an aggressive clinical behavior with 25% to 50% of patients presenting with metastatic disease. Most investigators agree that given the biologic similarities with Ewing’s sarcoma, PNET should be treated as a member of the Ewing’s sarcoma family of tumors. Standard therapy for the Ewing’s sarcoma family of tumors includes combined surgery and chemotherapy (vincristine, doxorubicin, cyclophosphamide, etoposide, ifosfamide). Given the highly aggressive nature of this tumor, adjuvant multidrug chemotherapy is encouraged by most Authors. Radiotherapy is recommended when resection is not possible at diagnosis or after chemotherapy induction in response to incomplete resection. Most recently, Jimenez and coworkers have reported a series (11 cases) of PNET of the kidney. Follow-up on 8 cases showed 4 lung and pleural metastases, 1 bone metastasis, liver metastasis, 2 local recurrences, and 5 deaths from disease (median time to death, 16.8 months). Adjuvant therapy included chemotherapy (8 cases), radiation (3 cases), and bone marrow transplantation (1 case). Three patients are alive without evidence of disease at 4, 16, and 64 months after diagnosis, respectively. These 3 patients received adjuvant chemotherapy, which included ifosfamide in 2 patients and cyclophosphamide in the other. Two of these patients also received adjuvant radiotherapy.

Conclusion

PNET of the kidney is likely to be more often recognized because of the increasing appreciation of this entity through broader application of immunohistochemistry and molecular biology. The diagnosis of renal PNET must be considered in young patients with renal neoplasm, particularly those with advanced disease at presentation. Achieving exact diagnosis has important clinical consequences because polychemotherapy may lead to tumor reduction or even complete remission.

References


È mancato Giorgio Baroldi, patologo cardiovascolare, protagonista per mezzo secolo delle ricerche e del dibattito sulla patogenesi dell’infarto miocardico e della morte improvvisa coronarica, simbolo del genio italiano nello scenario della medicina cardiovascolare internazionale (Fig. 1).

Nato a Piacenza nel 1925 si laureò in Medicina nel 1949 all’Università Statale di Milano, conseguendo la libera docenza in Anatomia Patologica nel 1959.


Morto il suo “mentor” prof. Pietro Radaelli, Direttore dell’Istituto, vista la povertà di risorse e lo scarso interesse dell’ambiente accademico italiano in tema di cardiopatologia, decise di migrare negli Stati Uniti a Washington presso l’Armed Force Institute of Pathology (AFIP) dell’Ospedale “Walter Reed”, nome del medico militare famoso per gli studi sulla febbre gialla. L’AFIP è una specie di Mecca dell’Anatomia Patologica mondiale, a tutti noi patologi ben nota per la serie di quaderni monografici su varie patologie d’organo, in specie neoplastiche. Baroldi ebbe la fortuna di incontrare e godere della fiducia del Dr. William Manion, Capo della Divisione di Patologia Cardiovascolare, un uomo straordinario dal punto di vista umano e professionale che, pur non interessandosi di cardiopatia ischemica (amava le cardiopatie congenite), mise a disposizione di Giorgio materiale di studio e mezzi per approfondire le sue idee sulla patogenesi dell’infarto miocardico. Nella tranquillità di Washington, dove si era trasferito con tutta la sua famiglia e dove sfrecciava con la sua famosissima fuoriserie suscitando con il suo fascino l’ammirazione e l’invidia di tutti, poté portare a termine la monografia “Coronary Circulation in the Normal and the Pathologic Heart”, pubblicata dall’AFIP, che rimane un capolavoro, una vera e propria Bibbia sul tema (Fig. 2), e che conservo gelosamente, memore dell’immortale affermazione del Cardinal Bessarione “In assenza
nel 1964, il Distinguished Achievement Award lo aveva ricevuto dal Presidente Ike Eisenhower e morte improvvisa in circostanze aggettive e disostruzioni coronariche.

I risultati di questo studio, successivamente condivisi da altri patologi americani quali Bill Roberts e Malcolm Silver, misero in dubbio la relazione causale fra trombosi coronariche e infarto miocardico, e contribuirono a tardare l’applicazione della trombolisi quale metodo di dissoluzione dell’occlusione coronarica acuta nella cura dell’infarto miocardico.

Con Malcolm Silver, Direttore prima della Patologia Cardiovascolare nel Nebraska e quindi al Banff Institute di Toronto, avviò un sodalizio di ricerche che culminerà nel famoso studio su 100 casi di infarto miocardico con una incidenza di trombosi coronariche del 34%.

La cosa gettò un certo sconcerto e sfiducia sulla catecolaminica, rivalutando i concetti di Selye sulle tensioni, diabete, obesità. Va ricordato che la morte improvvisa non fosse di tolemaica memoria, che insegnava a dubitare e a cancellarebbe la sua memoria. "nel estero che bisognava far rientrare e impiegare in una progettualità, tutta “made in Italy”. A Pisa, Luigi Donato aveva fondato poco l’Istituto di Fisiologia Clinica del CNS (la vera via Palisperna della Cardiologia Italiana dove fioriranno personalità quali Attilio Maseri e Antonio L’Abbate) e offrì a Baroldi di lavorare a Pisa. Tornò in Italia, riprendendo anche il ruolo universitario all’Università di Milano che non aveva mai lasciato. Si rimboccò le maniche per creare un laboratorio CNR di Patologia prima a Pisa e successivamente a Milano, all’Ospedale “Niguarda”. Il rientro coincise con la contestazione studentesca e non deve sorprendere che il suo modo di porsi, all’insegna del dubbio, in modo anticonformista e antibaronale, gli guadagnasse le simpatie degli studenti. Già nel 1973 lo avevo contattato, io neoiftica di una disciplina del quale avevo subito il fascino e che sarebbe diventata la ragione della mia vita professionale. Donato mi invitò a unirmi a lui, ma declinai l’invito, legato come ero già allora alla Universitas Patavina Libertas. Svilupparono ugualmente una collaborazione insieme ad Annalisa Angelini, che si tradusse in importanti contributi sulla genesi dell’aterosclerosi coronarica nei primi vent’anni di vita.


Nel 1989 Giorgio volle fortissimamente la creazione del Gruppo di Studio Italiano di Patologia Cardiovascolare, in grado di raccogliere tutti gli anatomo-patologi dedicati prevalentemente alla cardiopatologia, e ne divenne il primo Presidente.

Giorgio è stato un pioniere e lascia una traccia indelebile nella storia della cardiopatologia italiana e mondiale. Quando il 26 marzo 2000 a New Orleans venne insignito del “Distinguished Achievement Award” della
Society for Cardiovascular Pathology, mi venne affidato il compito di presentarlo. Ricordare i suoi contributi scientifici e le oltre 350 pubblicazioni, dalla cardiopatia ischemica alle miocarditi, dalla patologia da trapianto di cuore all’infezione da HIV e alla malattia di Chagas, era facile e scontato. Più arduo era presentare il Baroldi “umanista”.

Rilessi la sua “Autobiografia di una avventura scientifica. Storia naturale di una eresia” (Fig. 3), pubblicata nel 1989, in cui raccontava la sua scelta di diventare ricercatore “… La facoltà di medicina era quella che più di ogni altra studiava tutti gli aspetti dell’uomo. E l’uomo era e rimane il centro della mia attenzione. La scelta quindi fu facile e senza esitazioni. Altrettanto facile ma con qualche esitazione fu scegliere il cuore: curare il malato o ricercare le cause del suo male. Anche se attratto dal contatto umano della professione, prevalse l’esigenza del conoscere … la necessità di una visione il più possibile capace di enucleare la realtà dall’apparenza, quale solo la morfologia può dare … “. E concludeva così: “… La vita dell’eretico onesto … è come una guerra, bella ma scomoda. Bella per tutte le tensioni intellettuali, gli scontri concettuali, l’ansia di sentire o leggere fatti e idee nuovi che possono incrinare lo schema teorico che si viene costruendo, con la continua necessità di ricon- trollare, di cogliere l’errore sia altrui che nostro per tenere il discorso nella giusta traiettoria. Scomoda per la fatica e l’insonnia, per l’impossibilità di distrarre il pensiero, senza vacanza. Ma soprattutto dall’essere solo. La “scienza normale” ha il coro attorno che applaude e conforta. L’eretico ha solo se stesso e i suoi dubbi ingi- gantiti dalla sua solitudine fatta di distinguo, nel mezzo di una folla ostile e passiva con un continuo senso del-l’ingiusto. Intendo il termine “eresia” non nell’accezione di falsa dottrina (lo è per il credo corrente) ma di scelta – secondo il suo etimo – razionale di una dottrina antite- tica che può anche essere vera … tesi e antitesi saranno parti legittime di un processo logico nel quale non avrà più peso da che punto stia il torto o la ragione nell’inte- resse superiore della verità”.

Con Giorgio Baroldi se ne va un altro dei miei mentori (Italo Rizzi, Vincenzo Gallucci, Lino Rossi), ma il suo modello di ricerca nella morfopatologia clinica continuerà a vivere e ad ispirare il nostro Gruppo.

Gaetano Thiene
a nome del Gruppo di Studio Italiano di Patologia Cardiovascolare

Bibliografia


XII Self-Assessment Dermatopathology Workshop
Catholic University School of Medicine Rome, Italy

September 12th-15th 2007

300 slides of difficult or interesting cutaneous neoplasms (soft tissue and melanocytic tumors) will be available for a direct view at microscope for the entire September 12th and 13th. The faculty will then illustrate the most important single cases with transparencies September 14th and 15th.

Faculty: T. Mentzel, Dermatopathologische Gemeinschaftspraxis, Friedrichshafen, Germany (soft tissue tumors), G. Massi, Catholic University Medical School, Rome (melanocytic tumors).

Fee: 350 Euro

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