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HPV infection and the risk of penile cancer

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Primary malignant penile cancer is a rare disease. Penile cancer incidence varies among different populations, and is rare in most developed nations. In the United States, age-standardized incidence rates range from 0.3 to 1.8/100,000 [1]. Higher incidence rates are seen in underdeveloped countries, such as in Uganda (2,8/100,000), and in areas of Brazil (range from 1.5-3.7/100,000 inhabitants); the lowest incidence world-wide is reported in Israeli Jews (0.1/100,000 inhabitants) [1].

Penile cancer most commonly affects men between 50 and 70 years of age [2-4]. Younger individuals are also affected; approximately 19% of patients are less than 40 years of age [3] and 7% are less than 30 years [3 5].

Human papillomavirus (HPV) infection is the necessary etiologic agent for cervical carcinogenesis, with HPV infection in men significantly contributing to infection and subsequent cervical disease in women as well as to disease in men [6-8].

Many studies suggest an association between human papillomavirus (HPV) infection and penile cancer. The mechanism by which HPV leads to malignant transformation is likely mediated through two viral genes, E6 and E7, which are actively transcribed in HPV infected cells. The most recognized target of HPV E6 protein is TP53 [9], whereas the primary target of HPV E7 protein is RB1 and the related pocket proteins, p107 and p130 [10]. The E6 and E7 proteins bind to and inactivate the host cell's tumor suppressor gene products p53 and pRb (retinoblastoma gene) both of which are known negative regulators of cellular proliferation, leading to uncontrolled growth [11]. In cervical carcinogenesis, recombination between HPV and chromosomal DNA is frequent and likely necessary for progression, and DNA hypermethylation – specifically of the L1 gene – is a biomarker for cancerous progression [12]. Recently, Kalantari et al. [13] compared penile and cervical carcinoma with HPV 16 and HPV 18. They found numerous striking similarities: high HPV 16 methylation rates in penile carcinomas resemble those reported in cervical malignant lesions. They proposed that both penile and cervical carcinomas depend on chromosomal recombination as a necessary step in the etiological process. Their data support the causality of HPV infection in the etiology of penile cancer

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and suggest similar etiological and epidemiological parameters for HPV dependent cervical and penile carcinogenesis.

Partridge et al. recruited 240 male students, 18 to 20 years of age, at the University of Washington in Seattle to participate in a longitudinal natural history study of HPV infection [14]. At 24 months, the cumulative incidence of new infection of any genital HPV type was 62.4% (95% CI = 52.6-72.2%). The most commonly detected types were HPV-84 and HPV-16. In multivariate analysis, a report of a new sex partner during the prior 0-4 (hazard ratio [HR] = 2.0; 95% CI = 1.3-3.0) and 5-8 (HR = 1.8; 95% CI = 1.2-2.7) months and a history of smoking (HR = 1.6; 95% CI = 1.1-2.4) were associated with an elevated risk of HPV acquisition.

In men, productive HPV infection can result in simple condyloma acuminata, giant condyloma, or Buschke-Lowenstein tumor, mainly caused by PHV 6 and 11 [15]. HPV-associated penis intraepithelial neoplasia are found in the great majority of cases, but they are inconspicuous lesions caused by high-risk HPV types, especially HPV 16 and 18, histologically showing low, moderate, or severe dysplasia (PIN grades 1, 2 and 3) [16]. Less frequently, high-risk HPV infection can progress to penile carcinoma, also associated with HPV 16 in 16 to 100% of the cases, and HPV 18 in 1 to 55% of the cases [17].

In a systematic review of the literature, Dunne et al. [18] found a wide range (1-73%) of genitourinary HPV prevalence among men worldwide; 15 (56%) of these studies reported a prevalence of > 20%, which is similar to the HPV prevalence found among women (27%) [19]. Weaver et al. evaluated the distribution of HPV in men. Of 1323 samples tested (from 317 men), 215 (16%) were found to be positive for HPV DNA, including 28% from the foreskin, 24% from the penile shaft, 17% from the scrotum, 16% from the glans, and 6% from urine [20]. According to Giuliano et al., in heterosexual men, HPV detection was highest at the penile shaft (49.9%), followed by the glans penis/coronal sulcus (35.8%) and scrotum (34.2%). Detection was lowest in urethra (10.1%) and semen (5.3%) samples [21]. Nielson et al. tested 463 men for HPV at the glans/corona, penile shaft, scrotum, urethra, perianal area, anal canal, and in a semen sample. HPV testing by PCR and reverse line blot genotyping for 37 types was conducted on each of the specimens from the seven sampling sites [22]. When HPV results from any sampling site were considered, 237 (51.2%) men were positive for at least one oncogenic or nononcogenic HPV type, and another 66 (14.3%) men were positive for an unclassified HPV type. The types with the highest prevalence were HPV-16 (11.4%) and 84 (10.6%). External genital samples (glans/corona, shaft, and scrotum) were more likely than anal samples to contain oncogenic HPV (25.1% *vs*. 5.0%). HPV-positive penile shaft and glans/corona samples were also more likely to be infected with multiple HPV types than other sites.

A recent study reported the prevalence of HPV DNA in samples collected from exfoliated cells in men [23]. Overall HPV prevalence was highest in the penile shaft (52%), followed by scrotum (40%), glans/corona (32%), urine (10%), and semen (6%). The prevalence of any HPV infection in the glans/corona was significantly higher in uncircumcised men (46%) than in circumcised men (29%) (OR 1.96; 95% CI = 1.02-3.75). Uncircumcised men also had an increased risk of oncogenic HPV infection (adjusted OR 2.51; 95% CI = 1.11-5.69) and infection with multiple HPV types in the glans/corona (adjusted OR 3.56; 95% CI = 1.50-8.50) [23].

Nicolau evaluated the prevalence of HPV DNA in 50 male partners of HPV-infected women [24]. The brushings were HPV DNA positive in 35 (70%) of the men: 32% in the high-risk HPV group, 14% in the low-risk HPV group, and 24% in both groups. HPV detection per anatomic site was 24% in the glans, 44% in the prepuce internal surface, 30% in the distal urethra, 24% in the prepuce external surface, 12% in the scrotum, and 8% in the anus. Carestiato evaluated the prevalence of human papillomavirus infection determined by hybrid capture assay in 1,481 men [25]. The hybrid capture test (HCA II) is a non-radioactive, hybridization assay, designed to detect 18 HPV types divided into high and low-risk groups. The prevalence was 9.1% in the low-risk group, 9.7% in the high risk group and 7.4% with mixed infections, giving a total prevalence of 26.2%.

Castellsagué et al. [26] has shown a lower prevalence of penile HPV in men who have been circumcised (OR = 0.37; 95% CI, 0.16-0.85). In this large multination study, Castellsagué et al. found HPV in 19.6% of 847 uncircumcised men, but only 5.5% of 292 circumcised men. After adjustment for confounding variables, circumcision remained associated with less frequent HPV infection (OR 0.37). In healthy Mexican military men HPV prevalence was 44.6%, and OR for persistent HPV was 10 times higher in uncircumcised [27]. Nielson et al. [28] examined the association between HPV infections and circumcision at the glans penis/coronal sulcus, penile shaft, and scrotum in addition to the urethra, semen, perianal area, and anal canal in 463 men. Seventy-four men (16%) were uncircumcised. Adjusted odd ratios

(AORs) for any HPV genotype and circumcision were 0.53 (95% CI = 0.28-0.99) for any anatomic site/specimen, 0.17 (95% CI = 0.05-0.56) for the urethra, 0.44 (95% CI = 0.23-0.82) for the glans/corona, and 0.53 (95% CI = 0.28-0.99) for the penile shaft. These results suggest that circumcision may be protective against HPV infection of the urethra, glans/corona, and penile shaft. Auvert et al. analyzed the effect of male circumcision (MC) on the prevalence of HR-HPV [29]. In this study, 3274 uncircumcised men were recruited, randomized into 2 groups, and followed up. MC was offered immediately after randomization to the intervention group and after the end of the follow-up period to control group participants. Urethral swab sample was collected at the 21-month visit in 1264 participants, reported by randomization group. The urethra was chosen because the detection of HPV in this anatomical site is probably not affected by circumcision status. HR-HPV prevalence was 14.8% in the intervention group and 22.3% in the control group (prevalence rate ratio [PRR] = 0.66; 95% CI = 0.51-0.86; p < .002). The percentage of each of the 13 HR-HPV genotypes was lower in the intervention group than in the control group. The prevalence of multiple HR-HPV types was lower in the intervention group than in the control group (4.2% *vs*. 9.9%; PRR = 0.43; 95% CI = 0.28-0.66; p < .001). This controlled trial showed a reduction in the risk of HR-HPV infection among men after MC.

There is an association between the mean number of female sexual partners in the year preceding the study and the presence of HPV DNA. The higher the number of sexual partners the greater, the chance of acquiring and transmitting HPV. Castellsagué et al. [26] studied uncircumcised men who had had less than five sexual partners up to the time of the study and found that 12.5% of them were positive for HPV DNA, while among men who had had more than five sexual partners up to the time of the study the percentage of HPV DNA-positive subjects increased to 44.7%. Fransceschi et al. [30] found a highly significant association (p < 0.01) between the presence of HPV DNA and the number of sexual partners up to the date of the study, with 21.1% of men having less than 10 sexual partners being positive for HPV DNA, as opposed to 43.3% of men having more than 10 sexual partners. In another study by Rombaldi et al. [31], demonstrated that the greatest risk factor (p = 0.038) for acquiring HPV DNA was related to the total number of sexual partners up to the date of the survey, with men who had the highest number of sexual partners have the highest risk (p = 0.038) of being positive for HPV DNA.

The prevalence of HPV DNA in penile carcinomas ranges between 15% and 81% (Table I). Rubin et al. evaluated the prevalence of HPV DNA in different histological subtypes of penile carcinoma, dysplasia, and condyloma [32]. HPV DNA was detected in 42% cases of penile carcinoma, 90% cases of dysplasia, and 100% cases of condyloma. In this study, although keratinizing squamous cell carcinoma (SCC) and verrucous carcinoma were positive for HPV DNA in only 34.9% and 33.3% of cases, respectively, HPV DNA was detected in 80% of basaloid and 100% of warty tumor subtypes [32]. Cubilla et al. [33] reported detection of HPV 16 in 9 of 11 (81%) cases of basaloid and 3 of 5 (60%) cases of warty SCC of the penis.

Penile cancer, like cervical cancer, is caused by high-risk HPV, but penile cancer is 10 times less common than cervical cancer [34]. Many studies have shown the presence of HPV types 16 and 18 in penile carcinoma. In a case-control study in Uganda [35] the seropositivity to HPV-16, HPV-18, or HPV-45, the most common oncogenic types of HPV, was 46% among penile cancer cases and 12% among controls (OR 5.0, 95% CI = 1.4-17.2). In another case-control study done in the United States [36], positive HPV-16 serology was found among 24% of penile cancer cases and 12% of controls (OR 1.9, 95% CI = 1.2-3.2); 80% of penile cancer tissue specimens were positive for HPV-DNA. Heideman performed molecular and serologic analyses of HPV types on a series of 83 penile cancer squamous cell carcinomas (SCCs), and compared serological findings to those of age-matched male controls (n = 83) [37]. HPV DNA of mucosal and/or cutaneous types was found in 46 of 83 (55%) penile SCCs. HPV-16 was the predominant type, appearing in 24 (52%) of 46 of penile SCCs. The majority of HPV 16 DNA-positive SCCs (18 of 24; 75%) demonstrated E6 transcriptional activity and a high viral load. HPV 16 molecular findings were strongly associated with HPV 16 L1-, E6-, and E7-antibody. Furthermore, serologic case-control analyses demonstrated that, in addition to the association of HPV 16 with penile SCC, seropositivity against any HPV type was significantly more common in patients compared with in controls. Madsen et al. examined tissue samples of 37 penile SCC patients for the presence of HPV-DNA by PCR [38]. Twenty-four (65%) were hrHPV positive, and 1 (3%) was positive to a low-risk HPV type (HPV6). By far, the predominant HPV type was HPV16, which was detected in 22 (59.5%) of the 37 examined tumors, corresponding to 92% of the 24 hrHPV-positive tumors.

Guerrero et al. [39] detected HPV DNA by polymerase chain reaction in 4 of 10 patients (40%) with penile cancer, of which HPV 18 was present in 3 patients (75%), and HPV 16 and 18 in 1 (25%). In an examination of 30 specimens of penile cancer by polymerase chain reaction and in situ hybridization assays from 23 patients, the HPV-16 genome was found in 15 patients (65%), HPV-30 in 3 (13%), and HPV-6 or HPV-11 in 2 (9%) [40]. Bezerra et al. detected HPV DNA in 30.5% (25 of 82) samples of penile carcinoma in Sao Paulo, Brazil. HPV-16 was the most frequent type detected (13 of 25, 52%). Maden et al. [42] reported that, among 67 men with penile cancer who had tumor tissues available for HPV DNA testing, 49% were positive; the majority (69.7%) of which were type 16. Rubin et al. [32] found that the most common viral type identified in penile cancer was HPV 16, which was detected in 60% of HPV positive cancers. Pascual et al. [43] studied 49 patients with penile carcinoma. Thirty-eight patients of the 49 cases were positive for HPV (77.5%). HPV 16 appeared in 32 (84.2%) of the 38 positive cases and HPV 18 in 4 (10.5%). Lont et al. [44] detected high-risk HPV DNA in 29% of the tumors, with HPV 16 being the predominant type, accounting for 76% of high-risk HPV containing SCC. Scheiner et al. [45] evaluated the presence of HPV in penile cancer in Rio de Janeiro, Brazil. HPV DNA was detected in 72% of patients with invasive carcinomas and in 50% of patients with verrucous carcinomas. High risk HPV's were detected in 15 of 54 (27.5%) patients with HPV positive invasive tumors and in 1 of 4 (25%) patients with HPV positive verrucous tumors. The HPV 16 type was observed in 12 of 23 (52%) cases. Tornesello et al. [46] evaluated HPV genotype in 41 penile cancer biopsies from Italian patients. Among the 19 HPV-positive cases (46.3%) 2 viral genotypes were identified (HPV 16 and 18) with HPV 16 accounting for 94.7% (18 out of 19) of the infections. In this study, HPV-positive patients were significantly older (65.4 ± 7.3 *vs.* 58.1 ± 14.3); all patients under 50 years were HPV negative. Senba studied the relation between penile cancer and the prevalence of HPV genotypes in northern Thailand in 88 specimens of penile tissue [47]. In this study, an in situ hybridization (ISH) method was used to detect and localize HPV-DNA. Sensitive HPV polymerase chain reaction (PCR) procedure was used to detect and localize HPV-DNA, and DNA sequencing was used to identify the HPV genotype. HPV-DNA was detected in 53.8% and 81.5% of cases of penile cancer, using ISH and PCR, respectively. The most prevalent genotype was the high-risk HPV-18, found in 55.4% of the cases (as single infection in 32.3% and as multiple infections in 23.1%), followed by the low-risk HPV-6 found in 43.1% of the cases (as single infection in 24.6% and as multiple infections in 18.5%).

Table I. Prevalence of HPV-DNA in penile carcinomas.

REFERENCE	NO.	HPV-POSITIVE %
McCance [36]	53	51
Madsen [38]	37	68
Iwasawa [48]	111	63
Maden [42]	67	49
Chan [49]	41	15
Cupp [50]	42	55
Gregoire [51]	117	22
Picconi [52]	38	71
Rubin [32]	142	42
Guerrero [39]	10	40
Tornesello [46]	41	46
Scheiner [45]	80	72
Bezerra [41]	82	30
Pascual [43]	49	77
Giuliano [21]	303	65
Nielson [22]	463	65
Rombaldi [31]	99	54
Qiang [53]	28	61
Suzuki [54]	13	54
Senba [47]	65	81
Salazar [55]	54	65

Finally, Merck has announced recently that Gardasil, the vaccine that protects women from common strains of the human papillomavirus, has a 90% efficacy in preventing external genital lesions caused by HPV types 6, 11, 16 and 18 in men aged 16-26 years [48], suggesting that this vaccine may be efficacious in preventing infection and lesions from HPV in men. Future studies are warranted.

In conclusion, the incidence of penile cancer is low in developed countries, contrary to underdeveloped nations where the incidence could be as high as 3.5 per 100,000 inhabitants. The evidence suggests that circumcision may be protective against HPV infection of the urethra, glans/corona, and penile shaft. Similar to women, there is a direct proportional relationship between the number of sexual partners and the presence of HPV-DNA, and hence, a higher risk for developing a malignant pathology. In men with penile cancer, the prevalence of HPV infection ranges between 15 and 81%, and the most common oncogenic HPV genotypes found are 16 and 18.

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Lymph node dissection in squamous cell carcinoma of the penis

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Summary

Objective. The objective of this review is to present a structured report on the management of regional lymph nodes in squamous cell carcinoma (SCC) of the penis.
Methods. A Medline search with the key words "penile cancer" was performed between 2003 and 2008: 980 abstract have been found. Abstracts were organized according to subtitles of the present paper and links were used to find related articles of most interest.
Results. The lymphatic drainage of the penis was clarified both consulting old literature and new investigations. The diagnosis of nodal metastases was improved both with old and recent techniques, both in palpable and not palpable regional nodes. Indications and extent of regional lymph node dissection were clarified in the different clinical situations.
Conclusion. Improved technology and knowedge of the natural history of the disease allow hearlier diagnosis and improved medical care.

Keywords
Penis • Squamous cell carcinoma • Lymphatic drainage • Lymphnode diagnosis/staging • Lymphadenectomy

Penile cancer originates in the epithelium of the inner prepuce and of the glands. It is favoured by poor hygiene and it is associated to HPV infection in approximal 50% of cases. Histology and natural history are similar to squamous cell carcinoma (SCC) of horopharinx, female genitalia and anal canal. Tumour spread is to the inguinal and pelvic nodes, which are the regional nodes. Distant metastases are usually rare and late. Cure depends on control of the primary tumour and of regional nodes.
Cure of the primary tumour and early resection of regional lymph nodes metastases are the key for success in the management of penile cancer.
The aim of this paper is early detection and treatment of regional lymph node metastases.

Lymphatic drainage of the penis

Primary lymphatic drainage of penile cancer is to the inguinal nodes. Secondary drainage is to the pelvic nodes [1]. Daseler [2] in 1948 divided the inguinal nodes into four quadrants and a central circular zone in-

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tersecting vertical and orizontal lines at the junction of the saphenous vein with the femoral vein. Cabanas [3] in 1977 reported on lymphangiographic studies of the penis that "no drainage to the most inferior inguinal nodes and no direct drainage to the pelvic region was found". Subsequently, Catalona [4] in 1988 proposed a modified inguinal lymph node dissection reducing the area of dissection to the lymph nodes medial and superior to the saphenofemoral junction, but inguinal recurrences were reported in 15% of cases in the central and in the superior lateral zone. Recently, Leijte et al. [5] analysed the lymphatic drainage pattern of penile cancer in 50 patients using the SPECT-TC scanner. A total of 115 sentinel nodes and 182 higher-tier nodes were visualized. All sentinel nodes were located in both superior and central inguinal zones with prevalence in the medial superior zone. Catalona's mistake was not to dissect the superior lateral and central Daseler's zone [2 4].

Diagnosis of lymph node metastases

The point is that micrometastatic disease evades clinical diagnosis and up to 25% of patients with nonpalpable lymph nodes do harbour micrometastases [6]. On the other hand up to 30-50% of patients with palpable inguinal nodes will not have metastatic disease, but inflammatory lymph node swelling secondary to penile cancer. The most widely studied technique is that of *ultrasound guided fine needle aspiration* cytology: Saisorn et al. [7] reported a sensitivity of 93% and specificity of 91% for palpable lymph nodes. The problem is not palpable nodes. Common imaging techniques such as CAT scan or conventional MRI are unable to detect micrometatases [8 9]. Nanoparticle enhanced magnetic resonance imaging and PET/CT have been reported with results considered promising [10]. Scher et al used ^{18}F-FDG PET/CT, and detected 15 of 16 positive lymph nodes in 5 patients (sensitivity 80%, specificity 100%). In a recent update of the study, PET/CT identified 18 of 21 histologically positive lymph nodes (sensitivity 75%) [11].

Management of patients with no palpable inguinal nodes

Surveillance

Patients with low stage tumours and clinically unaffected inguinal nodes have in the past undergone surveillance strategies, i.e. follow-up. Indeed, the 2004 EAU guidelines "strongly" recommended this approach in patients with superficial and well differentiated tumours: Tis, Ta G1-2, T1G1 and T1G2 with superficial growth and no vascular invasion [12].

Prophylactic inguinal lymphadenectomy for cNO squamous cell carcinoma of the penis is not routinly performed even if saphenous vein sparing coupled with thick skin flaps reduced post operative complications significantly (> 50%) without compromising the recurrence rates [13 14].

For a long time, urologist have been oriented to look at *pathological risk factors* for metastases. In the INT series [15] nodal metastases were found in 100% of pT3-T4, in 82% in pT2 and 23% in pT1. In particular 16.5% of metastases were found in pT1G1 and 60% in pT1G2-3 No metastasis in CIS. Consequently, prophilactic LAD was performed in all patients pT2, pT3, pT4 and in pT1G2-3, and lymph node metastases were found in 60% of all patients at high risk of metastases at first presentation and in 100% at follow-up [15].

A better definition of cNO high risk patients could be given by *nomograms,* considering several risk factors which have been identified beside T and G categories: tumours thickness and front pattern of invasion [16] lymphatic and vascular embolization [17] perineural invasion [18], p53 [19]. All known prognostic factors have been put into a logistic regression model in order to construct a nomogram. It has been done by GUONE with Kattan's assistance [20]. This nomogram can estimate the risk of pathological inguinal lymphnodes according to 10 primary tumor variables. This nomogram showed a good concordance index (0.876) and good calibration but, surprisingly, grade 2 and superficial tumors resulted at worst prognostic features than grade 3 and infiltrating tumors [20].

Cabanas' [3] *sentinel node biopsy* (SNB) was a failure, but dinamic SNB (DSNB) how it was introduced by Horenblas [21] and Perdona [22] and improved by Kroon [23] and Leijte [24] is very promising. Following concepts developed in breast cancer and melanoma the technique of dynamic sentinel node biopsy (DSNB) was developed for penile cancer [24]. It is based on the identification of the lymph node which in the individual patient is the first drainage node (sentinel node). The concept assumes that there is a stepwise and orderly progression of the primarily involved node (the so called sentinel node) to secondary lymph nodes. More than one sentinel node can be involved. For identification of the sentinel node technetium-99m nanocolloid is injected around the penile tumour intradermally one day before surgery. Lymphoscintigraphy will iden-

tify the sentinel node(s) in the absolute majority of cases. Location is marked on the skin. In addition, shortly before the operation 1 ml of patent blue dye is injected around the tumor (or scar). The sentinel lymph nodes, detected intraoperatively with a gamma ray detection probe and patent blue dye staining, are dissected and removed. In case of positive findings, either on frozen section or definitive histology, a formal complete inguinal lymphadenectomy is performed.

The technique has been extensively studied only in few specialized centres. The group from the Netherlands Cancer Institute has repeatedly updated and published their results. They initially reported a high rate of false-negative cases [21]. Therefore they developed modifications of the technique and subsequently were able to report a markedly reduced false-negative rate of 4.8% [24]. Patients with positive sentinel nodes are candidates to groin lymph node dissection on the positive site. Criticism has been the role of a learning curve which requires a minimal amount of 20 procedures per year [25]. On site relapse of uncorrectly performed DSNB may happen.

Management of patients with palpable inguinal nodes

In patients with penile cancer, moderately enlarged palpable inguinal nodes which are not fixed may or may not signify metastatic disease. The rate of false positive nodes has been reported to amount up to 50% [6] but in more recent series it is much lower: 30% [18]. Ultrasound with fine needle aspiration biopsy is an excellent, speedy and easy way to find evidence of metastatic involvement. Of course this is only reliable in tumor positive finings. In suspected cases with tumor negative findings, the fine needle aspiration biopsy should be repeated. Dynamic sentinel lymph node biopsy is not reliable in this group of patients and should not be used [26 27]. Thus, in all tumor positive patients early lymphadenectomy should be performed [28 29] and bilateral radical lymphadenectomy is the standard procedure. In case of contralateral nonpalpable lymph nodes, surgical staging is recommended.

Radical inguinal lymphadenectomy

Radical dissection of the inguinal region is performed in the triangle of Scarpa: superiorly along the margin of the inguinal ligament, laterally along the sartorius muscle and medially along the adductor longus. The saphenous vein is divided at the apex of the Scarpa's triangle and at the confluence with the femoral vein. The anterior aspects of the femoral vessels are dissected, and at the end of the operation the femoral vessels may be covered by the sartorius muscle [30]. Thus, the lymph nodes in all five anatomic groups described by Daseler [2] are removed. The deep fascia is opened and the lymph nodes medial to the femoral vein are removed with the Cloquet node. A closed section drain is placed above the Sartorius muscle and brought out through a separate stab wound. It can be removed when drainage is down to 30-50 ml per shift. In the case of unilateral extensive disease, pelvic nodes may be approached prolonging the incision over the anterior superior iliac spine and dividing the abdominal muscles for approximately 5 cm. If a bilateral inguino pelvic lymph node dissection is planned, the operation may be performed through 2 separate inguinal incision and with a median sovrapubic incision for bilateral pelvic node dissection.

A significant morbidity has been described. Wound infection, skin necrosis, wound dehiscence, lymphoedema and lymphocele can occur [31 32]. Optimal skin handling and careful dissection of skin flaps is one of the most important aspects in prevention of complications. Skin rotation flaps and myocutaneous flaps are described for primary wound closure in advanced cases [33].

Modified inguinal lymphadenectomy

Catalona proposed a modified lymphadenectomy in order to reduce the morbidity and preserve the therapeutic benefit [4]. The main points are a shorter skin incision and limitation of the dissection (exclusion of the area lateral to the femoral artery and caudal to the fossa ovalis), preservation of the saphenous vein and no transposition of the sartorius muscle [4 32].

The morbidity of this procedure is reduced compared to radical lymphadenectomy [34 35]. Especially the incidence of skin flap necrosis, lymphoedema and deep venous thrombosis was remarkably decreased in a group of modified lymphadenectomy compared to a historical control group of radical lymphadenectomy. The rate of early complications was 6.8% (*vs*. 41.4%) and the rate of late complications was 3.4% (*vs*. 43.1%) for the patients with modified lymphadenectomy [36]. However, reducing the field of dissection increases the possibility of false-negative cases. The high false negative rate described by Lopes et al. [36] has to be discussed under the aspect of the recent findings concerning lymphatic drainage to the lateral superior Daseler zone,

which is not dissected in this approach [2 5]. Current knowledge of lymphatic drainage would suggest that a contemporary modified lymphadenectomy should dissect the central and both the superior Daseler zones of the inguinal region.

Video endoscopic inguinal and pelvic lymphadenectomy

This recently described technique is derived from laparoscopic surgery and has been evaluated only in small pilot studies [37 38]. It seems to carry a lower risk of skin complications but a higher risk of lymphocele formation (23%) compared to an open approach; the reported overall complication rate was 23% [39]. An assessment of this technique for its reliability is not yet possible.

Laparoscopic pelvic node dissection for bilateral pelvic lymph node removal following positive bilateral inguinal lymphadenectomy does have sense. But it must be beared in mind that this is not a staging pelvic lymphadenectomy as for prostate cancer, but it must be a radical bilateral pelvic lymph node dissection with the same template and accuracy for open surgery.

The role of pelvic lymphadenectomy

Cabanas and Leijte et al. did not detect direct lymphatic drainage to pelvic lymph nodes from penile cancer [3 5]. Thus, in cases of uninvolved inguinal nodes pelvic lymphadenectomy is not warranted.

On the contrary, if the Cloquet node is involved on one side, a contemporary pelvic linph node dissection is to be performed through an upword muscle-splitting incision. Patologic predictors for potential involvement of pelvic nodes in patients with positive inguinal nodes are the number of inguinal lymph nodes involved and extracapsular extent of metastatic disease [40]. Thus, pelvic lymphadenectomy may be necessary as a secondary procedure. In this case it can be performed though a midline sovrapupic extraperitoneal incision if a bilateral dissection is indicated. Since the rate of positive pelvic nodes has been reported to be 23% in cases with > 2 positive inguinal nodes and 56% for > 3 inguinal nodes involved [41-43], pelvic lymphadenectomy is recommended if 2 or more inguinal nodes are involved and/or exstracapsular extent in one inguinal node is seen. If very aggressive histological subtypes penile cancer are present (i.e. basaloid or sarcomatoid type) or strong expression of p53 is found, a pelvic lymph node dissection should be considered if any inguinal node is involved [44]. The boundaries of pelvic lymphadenectomy are: the common iliac vessels distally, the ileo-inguinal nerve laterally, the bladder and prostate medialy, the deepest part of opturator fossa and the bottom and the passage below the inguinal ligament to the groin inferiorly to assure that Cloquet node have been removed.

Morbidity of lymphadenectomy

Surgical morbidity is a significant problem after radical inguinal lymphadenectomy. Wound infection, skin necrosis, wound dehiscence and lymphocele have been reported in a high proportion of cases [4 45-48]. This has led to modified approaches and the development of new techniques.

However, it is questionable whether the morbidity reported for radical inguinal lymphadenectomy is as high today as it has been reported by historical series [46 49]. Improved intra- and postoperative management with better knowledge of the potential complications may contribute to a reduction of morbidity. Certainly, the technique of modified inguinal lymphadenectomy has resulted in a markedly decreased rate of complications (in a recent series 6.8% early and 3.4% late complications) [50]. In the study by Bouchot et al only 8/118 patients suffered complications and these were only minor [50].

However, undoubtedly inguinal lymphadenectomy remains a procedure prone to local complications and should be performed with care and diligent tissue handling. The prophylactic application of antibiotics is recommended [4 50 51]. There is a clear need for vacuum drains, while there are no clear rules for the duration of drainage [4 50 51]. Elastic stockings and/or pneumatic stockings should be used to reduce the chance of marked lower limb lymphoedema. Whether early ambulation and postoperative anticoagulation are useful or detrimental is discussed controversially depending on the school of thought of the respective authors [4 50 52].

DSNB is a sophisticated procedure of low invasivity. Reported complications rates of around 14-15% [53 54] compare favourably with those of radical inguinal lymphadenectomy in historical series. In their most recent series, Leijte et al. report a complication rate of only 5.7% [55]. Perdona et al. compared early complications (mostly seroma) in 40% and late complications (mostly lymphoedema) in 47% of patients following radical inguinal lymphadenectomy in a historical control series versus 14% early complications in DSNB in a more recent series [53]. The potential advantage of reduced mor-

bidity with DSNB seems less pronounced in comparison to modified inguinal lymphadenectomy A prospective controlled comparison between DSNB and modified or radical inguinal lymphadenectomy has never been done.

Conclusions

Lymphadenectomy remains an integral part of the management of patients with penile cancer since early inguinal lymphadenectomy improves prognosis in patients with minimally invasive disease. Surveillance strategies are recommended in very low risk patients only (pTis, pT1G1). In all other patients with clinically unaffected nodes nomograms or dynamic sentinel node biopsy are adequate for staging, but the last should be performed in oncological centres. Otherwise, a modified bilateral lymphadenectomy (avoiding the 2 lower Daseler's zones) should be performed for all pT1G2 or more invasive stages. Patients with documented tumor positive inguinal nodes should undergo radical inguinal lymphadenectomy. If more than two inguinal nodes are metastatically involved, pelvic inguinal lymphadenectomy is to be performed. Categories pN2 and pN3 patients should be offered adjuvant chemotherapy, as for head & neck cancer.

This way, the over all cure rate of penile cancer could increase from 50 to 80% in recent years [1].

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Priapism: pathophysiology and management

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Summary

Introduction. Priapism is defined as a persistent erection of the penis not accompanied by sexual desire or stimulation and can be a urological emergency. There are three different types of priapism: low-flow priapism, high-flow priapism and recurrent priapism. Unfortunately, clinical guideline does not establish a fixed set of rules for the treatment of this condition.

Methods. This review combined an analysis of clinicopathologic reports as well as a summary of clinical and basic science investigations on the subject to date. Moreover, the proposed pathogenesis of priapism is reviewed, and a survey regarding treatment modalities is given.

Results. The prognosis depends on the type of priapism and the amount of time that passes before therapeutic intervention. It is important to distinguish between these conditions as the treatment for each is different. Low-flow priapism is a compartment syndrome with intracavernosal anoxia, rising pCO2 and acidosis and urgent medical attention is mandatory to prevent erectile dysfunction. On the contrary in high-flow priapism intervention is not urgent and often unnecessary. Finally, recurrent priapism is a condition which is still not well understood and there is no standardised algorithm for the management of this condition.

Conclusions. Urologists should understand the importance of the disorder and be prepared to follow current principles of diagnosis and treatment to reduce or prevent its complications.

Keywords
Priapism • Penile erection • Erectile dysfunction • Ischemic priapism • Nonischemic priapism • Recurrent priapism • Urology

Introduction

Priapism is defined as a persistent erection of the penis not accompanied by sexual desire or stimulation, usually lasting more than 6 h and typically involving only the corpora cavernosa and resulting in dorsal penile erection with the ventral penis and glans being flaccid [1]. Rare exceptions with involvement of the corpus spongiosum and sparing of the cavernosal spaces have been reported [2]. In some cases, this condition can be an urological emergency and has many different causes. The recently published American Urological Association Guideline on the management of priapism sheds further light on the management of this potentially emergent condition, but the guideline does not establish a fixed set of rules or define the legal standard of care for the treatment of priapism [3].

Incidence in a population-based, retrospective cohort study was found to be 1.5 per 100,000 person-years and 2,9 per 100,000 person-years for men aged 40 years and older [4]. For men using intracorporal injec-

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tions to treat erectile dysfunction, the incidence ranges from 1% for the patients who receive prostaglandin E1 to 17% for patients who receive papaverine [5]. In children with sickle cell anemia (SCA), the incidence is reported to range from 6-27% [6 7]. In adults, the incidence increases up to 42% [8]. A different study in this population reports 89% of males with SCA will have an episode of priapism by age 20. The mean period is 125 min per event [9].

Methods

Evidence Acquisition

In broad terms, priapism may be regarded as an imbalance between arterial inflow and outflow. Burnett has recently reviewed the pathophysiology of priapism and suggested derangements in the diverse systems of regulatory control in erectile function. These deregulatory functions include possible overactivity of the veno-occlusive mechanism, arterial inflow, or neurogenic processes that can affect inflow or outflow. Conversely, the problem may be secondary to malfunction of the normal contractile activities of cavernosal smooth muscle cells [10].

The aetiology of priapism has been traditionally divided into primary or idiopatic and secondary to some other condition or disease process. In accordance with Pryor, for the purposes of clinical management, it is appropriate to distinguish between high-flow, low-flow and recurrent or stuttering priapism [11].

In this paper we aim to provide insight into the pathogenesis and treatment modalities of priapism.

Low-flow priapism

Low-flow, ischemic or anoxic priapism is the most common. The spectrum of clinical symptoms and signs is analogous to those found in other compartment syndromes. It is a prolongation of a normal painful erection and in the idiopathic form is frequently present on walking. During erection there is a relaxation of the smooth muscle in the cavernous arteries and tissue, this is associated with the increased arterial inflow and the decreased outflow of blood. The intracorporeal pressure may rise above mean arterial pressure and the inflow of blood then ceases. The persistence of erection and failure of detumescence, the persistent relaxation and failure of contraction of cavernous smooth muscle is associated with increasing anoxia, a rising pCO2 and acidosis [12]. The prolonged erection becomes painful after a variable length of time; therefore patients are warned to seek urgent medical attention for an erection lasting more than 4 hours. Early relief is associated with return of normal flaccidity, but more prolonged ischemia is associated with tissue oedema.

Histological studies have shown a defined pattern of pathology [13]. Interstitial oedema and thickening are present up to 12 h, by 24 h endothelial thrombocytic adherence is present and by 48 h necrosis of cavernosal smooth muscle cells and fibroblast proliferation has occurred, which may result in subsequent fibrosis and calcification.

In organ-bath preparations using isolated rabbit corpus cavernosum, Broderick et al. [14] data suggest that corporeal smooth muscle tone, spontaneous contractile activity and the response to α-agonists depends on the state of corporal oxygenation. These observations might be an explanation for the failure of locally administered α-antagonists to relieve ischemic priapism because of smooth muscle paralysis. Daley et al [15] documented a significant reduction in prostacyclin (PGI-2) production during hypoxia in rabbit corpus cavernosal cells, which was attributed to inhibition of the enzyme PG-2 synthase. In view of the role of PGI-2 as an inhibitor of platelet aggregation and white cell adhesion, these studies may provide some insight into the changes in corporeal haemostasis during ischemic priapism. Further studies have shown that re-oxigenation of these hypoxic rabbit cavernosal cells generates oxidative stress that interferes with the recovery of prostanoid production [16].

The production of nitric oxide (NO) in the corpus cavernosum is altered by hypoxia because NO synthase activity is affected by changes in oxygen tension [17]. During veno-occlusive ischaemic priapism, the entrapped pool of blood that is initially at arterial oxygenation becomes progressively hypoxic. The combined reduction of PGI-2 and NO expected under hypoxic conditions would favour platelet aggregation and white cell adhesion, leading to thrombus formation and tissue damage.

The end result of muscle necrosis after priapism is fibrosis which may be patchy in distribution and it is thought that TGF-beta has an important role in this process

Nieminen and Tammala found that in 21% the cause of priapism was the intracavernosal injection of a vasoactive agent that is injected [18]. Papaverine has been associated with a 5% risk at initial diagnostic testing, but a much lower risk when used as therapy [19]; most of these cases were in patients with psychogenic or neurogenic impotence.

Pohl et al. evaluated various etiologies for priapism in a study of 230 single case reports in the literature:

idiopathic causes comprised one-third of the cases, whereas 21% were attributed to alcohol abuse or medications [20].

The incidence range of priapism episodes is from 1% for those on PGE1 [21]. The most likely cause of prolonged erection, as a result of intracavernosal injection therapy, is overdosage.

Sildenafil is an orally active agent for the treatment of ED and in well-controlled trials the incidence of priapism appears extremely low, although it has been anecdotally reported in post-marketing surveillance studies.

Drug-induced priapism has been reported with a variety of medications, most commonly related to the antihypertensive drugs guanethidine, prazosin, hydralazine and the anticoagulants, including intravenous heparin, and the oral coumarins [22]. Generally priapism occurred after cessation of anticoagulant therapy, thus resulting in a rebound hypercoagulable state. Priapism has been reported with a variety of centrally acting drugs including the phenothiazines, paroxetine, fluoxetine and trazodone and cocaine may have synergistic effects in promoting priapism [23,24]. Cocaine-induced priapism has been reported in association with topical application to enhance sexual performance and intranasal and intracavernous injections. Priapism has also been reported in association with the recreational drug ecstasy [25].

Examples of neurologic etiologic factors include priapism in patients with degenerative stenosis of the lumbar canal, priapism secondary to cauda equine syndrome and herniated disk [26].

Trauma to the perineum, penis or groin, whilst usually resulting in high-flow priapism, can result in venous compression secondary to penile haematoma or oedema.

Different solid tumors have been associated with priapism, including both bladder and prostate cancer [27]. Malignant priapism has been reported as the initial presentation of metastatic renal cell cancer, gastrointestinal tract and rarely from testis, lung, liver, bone and sarcoma as a result of invasion of both the corpora and spongiosum. Malignant infiltration may obstruct venous drainage [28,29].

Idiopathic segmental thrombosis of the corpus cavernosum, total parenteral nutrition, appendicitis, amyloid and rabies have all been reported as a cause of priapism [30,31].

High-flow priapism

High-flow priapism is less common than low-flow priapism and can be classified as congenital due to arterial malformations; traumatic usually associated with penile, perineal or pelvic trauma, iatrogenic from post revascularization procedures directly to the tunica or idiopathic. The local blood gas tension in these patients is arterial and therefore the penis is not at risk of ischemia and subsequent fibrosis.

The onset of a post-traumatic, high-flow priapism may occur up to 72 hours after the injury. Pain is never as severe as in an ischemic priapism: the penis is often not maximally rigid and pulsation may be visible in the penis.

A mechanism for the pathophysiology of high-flow priapism is described by Goldstein's group in Boston: unlike a traditional arterovenous fistula, the condition is described as an arterial-lacunar fistula where the helicine arteries are bypassed and the blood passes directly into the lacunar spaces. The high-flow in the lacunar space creates shear stress in adjacent areas, leading to increased nitric oxide release, activation of the cGMP pathway and smooth muscle relaxation and trabecular dilatation. These authors also postulate that the delay in onset of high-flow priapism may be secondary to a delay in the complete necrosis of the arterial wall after the initial trauma or secondary to clot formation at the site of injury followed by the normal lytic pathway, which follow in a few days [32].

A rare case of high-flow priapism is Fabry's disease, which may be caused by an unregulated high arterial inflow [33].

Recurrent priapism

Recurrent or stuttering priapism is associated with the hyper-viscosity syndrome, the commonest of which is sickle-cell disease which still ranks as the most frequent cause of priapism in children [34]. In a boy with sickle cell disease the incidence of priapism is of 18-27% [35]. This poorly understood condition is uncommon and not confined to men with sickle cell disease. The erection is usually during sleep and detumescence does not occur upon waking. These erections usually do not become painful for about an hour. Serjant et al. described "stuttering" nocturnal attacks in 42% of Jamaican adults with homozygous sickle-cell disease.

Recurrent episodes may result in a markedly enlarged penis with fibrotic corpora, which may later lead to ED.

Other haemoglobinopathies, including the rare unstable haemoglobin Hb Olmsted and thrombophilia erythropoietin therapy, the leukaemias and myeloma have also been associated with priapism [36].

Discussion

Diagnosis of priapism

A thorough history and physical examination are prerequisites to diagnostic accuracy. The fundamental aim of the initial phase of assessment is to distinguish arterial from ischemic priapism. The sexual and medical history should especially focus on medications, trauma and predisposing comorbidities. Presence or absence of pain is a fairly reliable predictor of low-flow versus high-flow priapism, respectively. Absence of pain in arterial priapism frequently results in less patient anxiety and discomfort as compared with veno-occlusive priapism. Consequently, those with arterial priapism may present days or even weeks after the original injury [37].

Physical examination of the penis is critical and typically reveals firm corpora cavernosa and a soft glans, indicating sparing of the corpus spongiosum in low-flow priapism. Findings in high-flow states usually reveal a partial to full erection and sparing of the corpus spongiosum in most cases [38].

General diagnostic test include urine toxicology screening for psychoactive drugs and metabolites of cocaine. It has additionally suggested reticulocyte count; urinalysis; complete blood count; platelets and differential white blood cell count.

Urologic management of priapism requires assessment of corporal blood flow status with corporal aspirate, visual inspection by color and consistency or corporal blood, and blood gas analysis including pH, pO2, and pCO2.

Low-flow priapism is suggested by finding low oxygen tension, high carbon dioxide and low pH in the blood gas analysis of the aspirate [39]. When a high-flow state is suspected based on the bright red appearance or blood gas analysis of the corporal aspirate, colour Doppler ultrasound is indicated to identify the arterial sinusoidal fistula (Fig. 1). For high-flow priapism, angiography is useful to identify a local bleeding site (Fig. 2).

Blood gas measurements of pH can give an indication of the urgency based on the degree of acidosis. A pH less than 7.10 reflects more aggressive management options and should be sought quickly in that the tissue is a risk for necrosis.

Penile colour Doppler ultrasound is not invasive, does not expose the patient to ionizing radiation and can reveal important information regarding the location of arterial injury in high-flow priapism by recording the turbulent flow that permeates the erectile tissue. Vascular lacuna is evident during selective pelvic angiography when the contrast medium, injected through the pudendal artery, spread the cavernous body and has the radiologic appearance of an arterial fistula [40]. The colour Doppler ultrasound is sensitive as angiography for the diagnosis of high-flow priapism. More specifically, penile colour Doppler ultrasound had a sensitivity of 100% and specificity of 73% with a predictive value of 81% for a positive test and 100% for a negative test [41].

Treatment of priapism

Therapeutic options for low-flow and high-flow priapism are essentially different, reflecting profound differences in etiology and pathophysiology. While ischemic priapism is a urological emergency that must be

Figure 1. In this patient spectral analysis shows the typical waveform pattern of an arteriosinusoidal fistula.

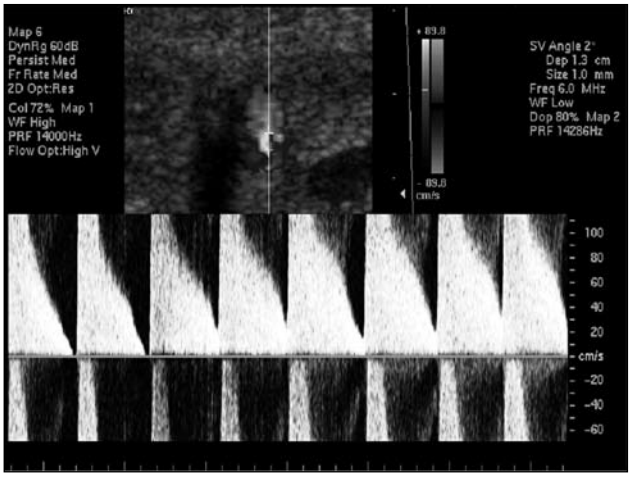
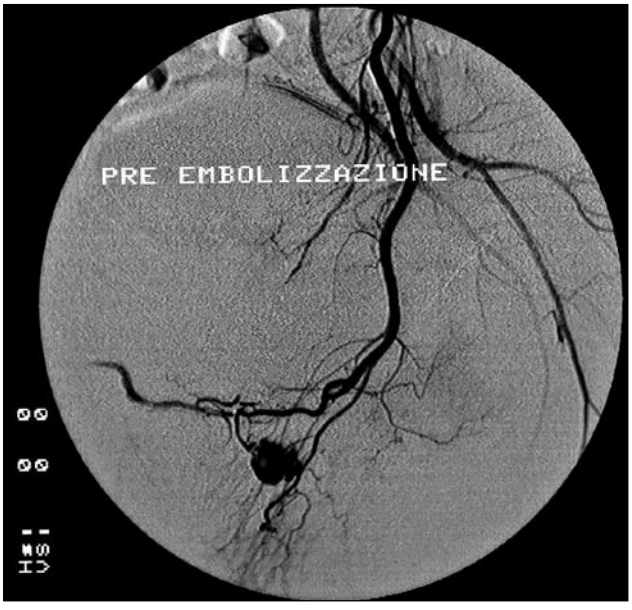


Figure 2. Arterious laceration with a cavity within the cavernosal tissue.



treated immediately, also using invasive procedures, patients with high-fl ow states are in general at low risk of developing irreversible erectile dysfunction and can be managed more conservatively.

Treatment of low-flow priapism

Therapy of low-flow priapism is based on the underlying cause and will typically follow a pattern of least invasive to more invasive procedures.

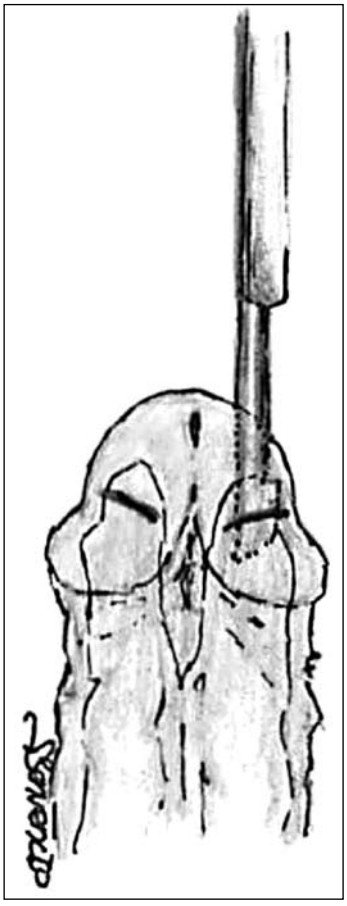
Any primary factors involved in the cause of the priapism should be addressed and treated. Pain and anxiety also require therapy, which includes use of parenteral opioids and an anxiolytic if indicated. Ice and elevation are also components of the initial conservative therapy. A penile dorsal nerve block utilizing local anesthesia, circumferential penile block, subcutaneous local penile shaft block and oral conscious sedation for pediatric patient may be of benefit to control pain [42].

For patients with low-flow priapism of relatively moderate duration (approximately 4 hours) penile aspiration and irrigation with saline remain standard first line management strategies.

For patients with extremely prolonged low-flow priapism, oral terbutaline, an oral β-adrenoreceptor agonist, in a dose of 5-10 mg has been advocated as a treatment with a response rate to 36% of treated patients who had prostaglandin induced priapism as well as therapy for other causes [43]. Terbutaline also can be given subcutaneously in does of 0.25-0.5 mg and can be repeated in 15-20 min. Oral pseudoephedrine, α-adrenoreceptor agonist, 60-120 mg orally has been suggested and used as therapy for priapism due to intracavernosal injected agents, but efficacy is not well studied. Treatment with injections into the corpus cavernosum of alpha adrenergic receptor agonists after aspiration would be the next therapy after terbutaline. Phenylephrine, 10 cc, which corresponds to a dose of 200 µg, is injected into the penis after aspiration. Frequent blood pressure measurements and preferably ECG monitoring are required throughout and failure to respond may require a second injection of 200µg and a final dose of 500 µg. Alternatively, epinephrine can be injected in 1-3 cc boluses up to 10 cc [44]. Methylene blue has been shown to be useful as an alternative to alpha agonists, with a mechanism felt to be related to inhibition of cyclic GMP, which in turn inhibits smooth muscle relaxation [45]. Intracavernosal injection with 50 mg of methylene blue followed by aspiration and penile compression for 5 minutes. Transient penile burning and blue discoloration lasting for about 3 days were the reported side effects [46].

If these relatively simple measures fail, surgical intervention is required. A variety of techniques has been described, including The Winter procedure using a Trucut needle (Fig. 3) and the Ebbehoj using a pointed scalpel blade to create a shunt between the glans and corpora cavernosa. El-Ghourab technique is a more invasive procedure that involves excision of a small disk of tunica albuginea. These techniques fail in about a third of patients.

Figure 3. Winter procedure using a Trucut.



Use of intracavernosal thrombolytic medications, including tissue plasminogen activator, has been recently described, although the efficacy is uncertain and long-term prognosis are lacking [47].

In a men with a late presentation of a low-flow priapism – more than 72 hours – consideration should be given to the implantation of a penile prosthesis.

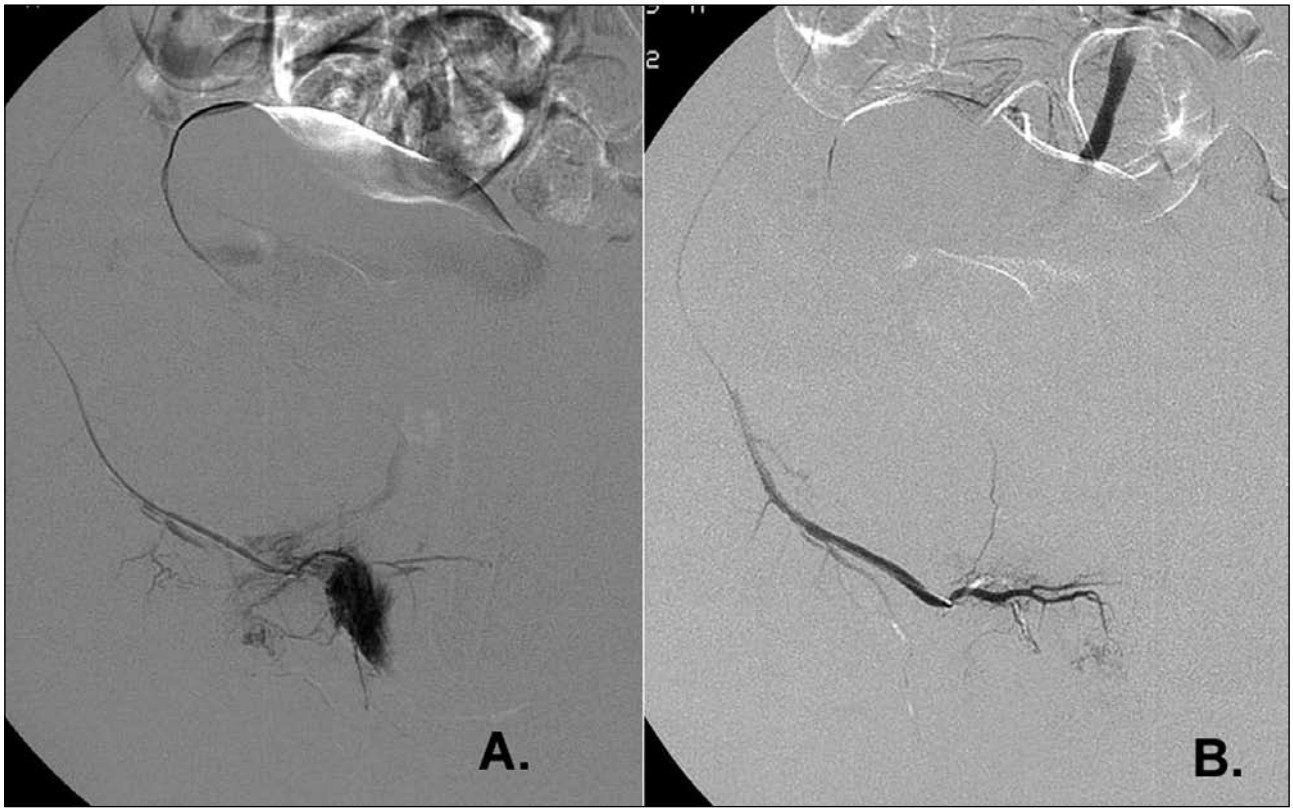
The treatment of sickle cell priapism requires more disease-specific treatment, including oxygenation, hydration, alkalinization, exercise, analgesia and exchange transfusion. Anecdotal evidence supports the use of oral therapy with hydroxyurea and hydralazine [48]. Etilefrine is an oral α-adrenoreceptor agonist that in the form of maintenance therapy may help prevent further attacks, with little effect on systemic blood pressure [49].

Surgical spinal decompression has been recommended to alleviate priapism associated with lumbar spinal stenosis.

Treatment of High-Flow Priapism

The clinical history and initial investigation, coupled with selective angiography, should confirm the diagnosis of high-flow priapism. The initial treatment should be observation. This approach is based on the

Figure 4. a) Pre-embolization location of the arterial-lacunar fistula. b) Absence of flow after the embolization with absorbable materials.



finding that expectant management results in spontaneous resolution in 62% of the report cases. The others cases are best managed by an interventional radiological procedure to embolize the responsible vessels using either autologous blood clot, silver coils, Geolfoam polyvinyl alcohol or N-butylcyanocyalate although several attempts may be necessary (Fig. 4). Open surgical ligation of the responsible vessels using intraoperative ultrasonographic guidance may be used when conservative and minimally invasive methods have failed.

Complications

Early complications typically result from injection of α-adrenergic agents and include headaches, palpitation, hypertension and cardiac arrhythmias. Vital signs should be monitored during this phase of therapy. Additional adverse events include urethral injury and urethrocutaneous or urethrocavernosal fistula from aggressive needle decompression, bleeding and infection [50]. Rare cases of gangrene of the penis after corporospongiosal shunt have been reported.

Prognosis

Impotence rates from 35-60% have been reported when priapism persist for 5-10 days, respectively. When the priapism has been ongoing for over 24 h, treatment with aspiration alone is often unsuccessful and will usually require irrigation and often injection. Treatment should be initiated within 12 h of the onset of symptoms to avoid long-term dysfunction and irreversible infarction, with the corollary being the earlier the resolution of symptoms, the better the long-term prognosis.

Conclusion

Current management strategies suffer from a poor understanding of the pathophysiology, especially at the molecular level. The traditional treatments are based more on empirical rather than evidence-based knowledge. Therefore, it is critical to understand priapism from a molecular level, to formulate treatment strategies and to establish rational prevention strategies. When the physician first diagnoses which type of priapism exists, distinguishing the type of priapic event is paramount in order to choose the correct treatment options. Until recently, we had not sufficiently understood the pathogenesis of this erectile disorder and therefore, could not effectively manage its pathologic consequences of erectile tissue damage and erectile dysfunction.

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Confounding factors in the evaluation of alpha-feto-protein plasma levels in patients with testis cancer

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Summary

Objective. To decribe the clinical conditions characterized by an increase of alpha-feto protein plasma levels that might confound the clinician in the diagnosis and follow-up of adult patients with testis cancer.

Materials and methods. We performed a brief review of the benign and malignant causes of alpha-feto protein plasma level elevation in the adult introducing few aspects regarding the physiological secretion and function of this protein.

Results. The clinical management of alpha-feto protein plasma levels increase in patients previously treated for testis cancer has to consider carefully all the other known causes of its elevations, from non malignant diseases (such as acute and chronic hepatitis) to malignancy of different tissues other than testis cancer, that are mainly from gastrointestinal tract but that have been reported also in breast, kidney and prostate cancer. These aspects are also more important in the absence of clinical conditions compatible with any recurrence of previously treated testis cancer.

Conclusions. Alpha-feto protein is a well known useful serum marker for the diagnosis and follow up of patients with testis tumors. However many different benign and malignant diseases are characterized by elevation of alpha-feto protein plasma levels without germ cell tumor growth and possibly confounding the physician. Then all the clinical conditions characterized by serum alpha-feto protein increase, other than germ cell tumors, have to be taken into account before assuming that elevations of plasma levels of this marker reflect the activity of testicular cancer in order to avoid unnecessary and dangerous treatments.

Keywords
Alpha fetoprotein • Germ cell cancer • Testis • Liver • hCG

AFP is a fetal protein that was first identified in 1956 during electrophoretic experiments on plasma proteins of infants [1] in the α1 position next to serum albumin. Alpha-fetoprotein (AFP) is a single chain glycoprotein of 590 amino acids with a Mw of about 67,000 Da. The AFP and albumin gene, arosed through duplication of an ancestral gene 300-500 million years ago, together with the gene for alpha-albumin or afamin and vitamin D-binding protein constitute the albumin multigene family.
AFP is produced by the fetal yolk sac, liver and, to a lesser extent,

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by the gastrointestinal tract and the kidney [2,3]. Various benign and malignant conditions can produce elevations of AFP in adults. After birth AFP secretion decreases dramatically so that in normal adult its plasma levels are close to zero albeit still detectable depending on residual production by the liver [3,4]. When monitoring serum levels of AFP, the age of the patient must be taken into consideration as the normal values do not apply to young children and to pregnant women where AFP plasma levels assume other clinical meanings that are not the matter of the present review.

Clinical interest in AFP raised after the observation that secretion of this protein, that is dramatically reduced in adulthood, can resume in patients affected by certain tumors together with non neoplastic disorders [3,5-7].

Together with human chorionic gonadotropin (hCG), AFP is the main tumor marker used to monitor testicular cancer, ovarian cancer and malignant teratoma wherever located in the body.

Any increase of AFP plasma levels might indicate tumor growing so it is necessary to know any possible confounding factors in order to avoid mistakes in considering its elevations as a indirect confirmation of germ cell tumor recurrence after treatment.

AFP Secretion

In human embryo AFP secretion starts around the 30 day after conception [8]. Around the 11-12 week of gestation AFP synthesis proceeds mainly by fetal hepatocytes. At 14 week of gestation AFP plasma levels reach the maximum (3 mg/ml) being the most represented protein in fetal serum. Then AFP plasma levels decrease progressively reaching their minimum at term, being as low as 30-100 µg/ml and then dropping dramatically to nearly undetectable levels just after birth and maintained to these low levels throughout life (less that 10 ng/ml).

Physiological role of AFP

The physiological functions of AFP are not well known. Given its similarity in physical properties with albumin and the fact that their presence within plasma is inversely related during the different phases of development, some authors have considered AFP as the fetal counterpart of albumin and it has been proposed that this protein has a role in the regulation of plasma osmotic pressure and as a carrier transport protein. Furthermore AFP seems to have a role in the immune modulation for the protection of fetus from potentially harmful maternal anti-fetal reactivity. It has been also suggested that AFP promotes the initiation of T-helper cell tolerance [9] thus helping to maintain the fetus as an allograft in a genetically incompatible environment. These suggestions seem to be confirmed by the observation that the administration of anti-AFP antibodies to pregnant mice and rabbits is abortogenic [10]. Finally a role in cell proliferation and differentiation together with different growth factors has been suggested for AFP [11-13].

AFP and testis cancer

Testicular cancer is the most common malignant neoplasm in young men accounting for about 1% of cancer in the male [14] and with an overall incidence of 7.5 cases for 100.000 although with some differences between countries [15]. About of 95% of testicular tumors origin from germ cells [16] while stromal testicular tumors are very rare [17].

Germ cell tumors are the most common types: seminomas account for at least half of all testicular tumors; embryonal carcinomas are the most common testicular tumor in boys while choriocarcinomas usually occur later, usually in the second and third decades of life; teratomas, second in frequency to embryonal carcinomas in boys, frequently contain a combination of germ cell types. Gonadoblastomas, usually occurring in dysgenetic testes, contain germ and stromal cells. Stromal tumors are constituted by Leydig or Sertoli cells and can be also of mixed origin. While Leydig and Sertoli cell tumors can secrete sexual steroids, germ cell tumors can secrete several different tumor markers in the bloodstream.

AFP and hCG are the most important tumor markers in germ cell cancer with an important diagnostic and prognostic role and must be always determined in all cases of germ cell tumors. An increase in these serum levels markers during the treatment and follow-up can indicate progression, recurrence or residual tumour.

AFP is one of the most used serum markers for germ cells tumors and in particular of tumors containing yolk sac elements [18].

AFP is always normal in pure seminomas while increases in 50-70% of patients with non seminomatous germ cell tumors, particularly in those containing elements of yolk sac or endodermal sinus components [8]. Below the age of 15 years, about 90% of testicular germ cell cancers are yolk sac tumours and in virtually all these patients serum AFP is elevated at diagnosis and is an excellent indicator of the response to therapy and

disease status. Tumors that histologically appear as seminomas but that have elevated serum levels of AFP should be treated as nonseminomas. AFP has a half-life of 5 days and degradation curves have to be followed after orchiectomy to assess for residual disease.

During the treatment or follow-up of patients with germ cell tumor, we can find several different clinical situations characterized by elevation of serum AFP without germ cell tumoral growth as detailed below.

Clinical significance of serum AFP elevation in adults

After birth AFP plasma levels usually fall within 8 to 12 months to concentrations lower than 10 ng/ml that are maintained throughout adult life. The rise of AFP plasma levels above normal range in adulthood is present in many different malignant and non malignant diseases as reported in Table I.

The highest AFP concentrations are encountered in patients with hepatocellular carcinoma [19]. AFP is abnormally secreted in approximately 70% of hepatocellular carcinomas (HCC). The diagnostic and prognostic role of AFP plasma levels in the diagnosis and management of this disease is well known and confirmed in many different studies being frequently measured in clinical practice during the course of treatment of HCC based on the hypothesis that AFP reflects the tumor activity [20].

Other tumors have been associated with elevated AFP plasma levels as well as pancreatic cancer (23%), gastric cancer (20%), bronchial cancer (7%), colorectal cancer (5%), and with lower frequency in cancer of the esophagus, small bowel, gallbladder, breast, endometrium, kidney, prostate and metastatic liver disease (19 and references therein).

As easily derived, in all these cases elevated AFP plasma levels hamper its use as a specific serum marker for the detection of germ cell tumor and/or its recurrence after treatment.

Among non tumoral diseases characterized by an increase in AFP plasma levels the majority regard almost exclusively liver diseases as well as acute viral hepatitis, chronic hepatitis, liver cirrhosis, alcoholic and drug induced liver damage, liver trauma and acute liver necrosis [19 21] (Table I).

We have to mention here a peculiar condition characterizing some adults that show persistent elevations of AFP without any clinical explanation. In these condition the so called hereditary persistence of AFP should be considered [19]. This is a clinically benign genetic condition that has an autosomal dominant inheritance pattern characterized by continued expression of the AFP gene in adult life. It can be easily confirmed, when possible, by analyzing AFP plasma levels in family members. Affected subjects had mean serum AFP levels 20-fold higher than normal healthy subjects.

Another particular condition regards the false positivity of AFP plasma levels observed after intensive chemotherapy for germ cell cancer that is due to drug induced liver damage. To this regard it has been proposed the use of the AFP binding ratio to concanavalin A (a lectin) as highly sensitive and specific tool to distinguish between AFP derived from non germ cell tumor and AFP derived from damaged hepatocytes [22].

Table I. Causes of serum alpha-fetoprotein elevation in adult.

NON MALIGNANT		
	Acute liver hepatitis	
	Chronic hepatitis	
	Liver cirrhosis	
	Alcohol and drug induced liver damage	
	Hereditary persistence of AFP	
MALIGNANT		
	Hepatocellular carcinoma	
	Non germ Cell Tumor	
	Germ cell Tumor	
	Other cancer:	
		Pancreas
		Stomach
		Broncus
		Colo-rectus
		Esophagus
		Small bowel
		Gallbladder
		Breast
		Endometrium
		Kidney
		Prostate
		Metastatic liver disease

Conclusions

AFP, together with other serum markers, is a well known useful clinical tool for the diagnosis and fol-

low up of patients with germ cell tumors. However many different benign and malignant clinical conditions may present serum elevation of AFP without germ cell tumor growth and possibly confounding the clinician. Then all clinical conditions characterized by serum AFP increase, other than germ cell tumor, have to be taken into account before assuming that the elevations of AFP reflect the activity of this malignancy.

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5-years experience with Video Endoscopic Inguinal Lymphadenectomy (VEIL): learning curve and technical variations of a new procedure

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Summary

Introduction. Video endoscopic inguinal lymphadenectomy (VEIL) was described in clinical arena 5 years ago to duplicate the open template reducing morbidity and without compromising the oncological control. The objective of this report is to review the technical evolution and variations described and report the learning curve aspects obtained by pionners in this period.

Matherial and methods. It was performed a search in important data bases including MEDLINE, LILACS, CANCERLIT and GOOGLE considering as key words video endoscopic inguinal lymphadenectomy, penile cancer, inguinal lymphadenectomy, laparoscopy. The technical variations for endoscopic approach described was resumed and critically analysed. Personal experience was utilized to ilustrations of surgical steps and to describe the learning curve data.

Results. All technical variations described to open surgery were safe and feasible by endoscopic approach. In terms of reprodutivity preliminary results of a ongoing word wide survey identified that 11 centers already performed VEIL. Operative time of VEIL is greater in the learning curve compared to the the open procedure. When comparing the first 10 and the last 12 procedures there was a small reduction in mean operative time (120 to 105 min), but there were no differences in complication rate.

Conclusions. VEIL is a procedure in your infancy. Reduced morbidity and good midterm oncological results are important arguments to growing acceptance of this new minimally invasive option to manage inguinal lymphnodes in high risk penile cancer patients.

Keywords
Penile cancer • Inguinal lymphadenectomy • Laparoscopy • Endoscopic procedures • Surgery

Introduction

Penile cancer is a rare disease at developed countries. A recent epidemiologic study shows that in some Northest states of Brazil as Maranhão this neoplasm can be the 2nd cause of malignant disease in men [1].

After local invasion inguinal lymphonodes are the first place prone to dissemination. In patients with unpalpable nodes 20-30% already have assintomatic metastasis [2].

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When the dissemination is still at the inguinal nodes, the disease is potentially curable by radical inguinal surgery [3]. Untreated lymphonodal disease is either an important cause of morbidity or an important predictive factor for cancer specific and overall survival [2 3].

Despite of the surgical benefits of prophilatic inguinal dissection at the time of diagnosis [4 5], contemporary series shows that the extended inguinal lymphadenectomy surgical morbidity is more than 50% [6 7].

In the last 20 years some alternatives were proposed attempting to reduce surgical morbidity after inguinal lymphadenectomy based on limited lymphonode templates [8-10]. Although potentially less invasive, this options had some drawbacks concerning cancer control, and inguinal recurrence ranging 5-15% at the follow-up occurred in all of this techniques [11-13].

Video endoscopic inguinal lymphadenectomy (VEIL) was described in clinical arena 5 years ago to duplicate the open template reducing morbidity and without compromising the oncological control [14].

The aim of this report is to review the technical evolution and variations described herein and report the learning curve aspects in a 5-year period.

Hystorical aspects of VEIL development

The concept of endoscopic inguinal dissection was proposed by Bishoff et al showing the feasibility dissecting 2 cadaveric models in 2003 [15-17]. This authors try to operate a patient and they did not complete the operation due to lymphonode fixation to femoral vessels preventing a safe ressection.

Based on this report, our initial protocol did not include patients with palpable inguinal lymphonodes. VEIL was also based on other endoscopic surgeries described in Cardio-vascular [18], Plastic [19] and Gynecologic surgery [20].

After some modifications of Bishoff's procedure, the first case in clinical scenario was successfully operated at ABC Medical School, São Paulo, Brazil in 2003 [14].

The first 3 cases was presented at the AUA annual meeting podium section in 2005 [21].

Our first study protocol was designed to test feasibility of lymphonode ressection and evaluate surgical morbidity [22]. Beetwen 2003 and 2005, ten patients were prospectively included on this study. They were diagnosed with penile carcinoma with no clinical inguinal lymphatic dissemination at the time of diagnosis. All patients had high risk pathological factors for inguinal dissemination such as pathological stage > pT1, histological grade > 1 or micro vascular or lymphatic embolization [2 15]. Patients underwent previous penectomy and, 1 month after the initial surgery, were selected to inguinal procedure based on the pathology diagnosis of specimen. After patients underwent bilateral inguinal lymphadenectomy following our protocol:

1. classic open inguinal lymphadenectomy at one leg – standard procedure;
2. VEIL at the other leg – study group.

Comparison of VEIL with open procedure in this preliminary study showed a reduced overall complication rate of endoscopic thecnique (20 x 70%) specially related to skin events The same number of nodes was removed comparing the approachs.

A second study was designed to test if VEIL could promote the advantages related to minimally invasive procedures [23]. Results of this study suggested that a reduced hospital stay and a faster recovery could be achieved in more 6 patients when bilateral VEIL was applied. The feasibility of VEIL in N1 patients was adictionally proved. There were no recurrence in a mean time follow-up of 36 months.

Technical aspects

Conventional VEIL (superficial and deep inguinal dissection) [17 21]:

1. patient positioning and inferior member preparation. Patient was positioned in supine position with thigh abduction. The video system was placed at the opposite side next to the patient's waist (Fig. 1);
2. initial access and surgical team positioning. A 1.5 cm incision was made 2 cm distally to the lower vertex of the femoral triangle (Fig. 2). Scissors and digital maneuvers were used to develop a plane of dissection deep to Scarpa's fascia (Fig.

Figure 1. Patient positioned in supine position with thigh abduction and external rotation. The video system was placed at the opposite side.

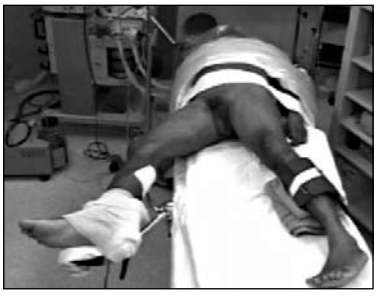


Figure 2. Boundaries of the femoral triangle. Inguinal ligament superiorly, medial border of sartorius muscle laterally and lateral border of adductor longus muscle medially.

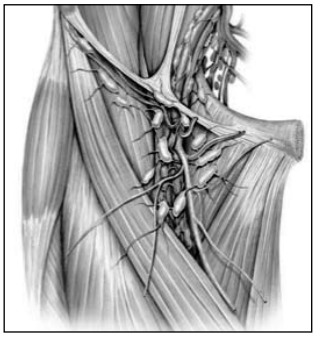
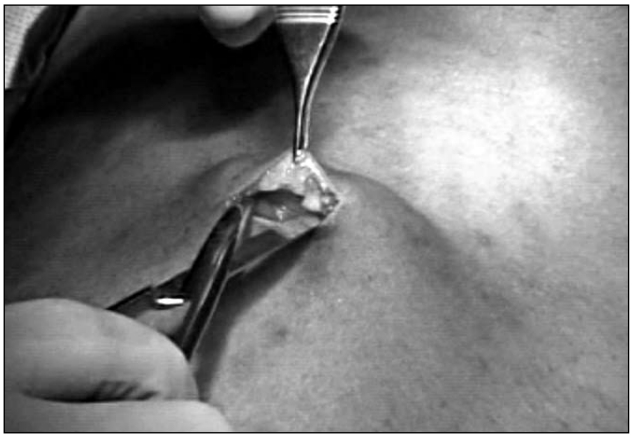


Figure 3. Skin incision is made 2 cm distally to the lower vertex of the femoral triangle. Dissection plane is developed by scissors and digital maneuvers deep to Scarpa's fascia.

Figure 4. Port sites for right side procedure. Hasson trocar is inserted in a incision 2 cm distally to the lower vertex of the femoral triangle. A 10 mm and a 5 mm trocars are placed 6 cm medially and 6 cm laterally to the apex of the triangle, respectively. The dissection area is insuflated and transilluminated.

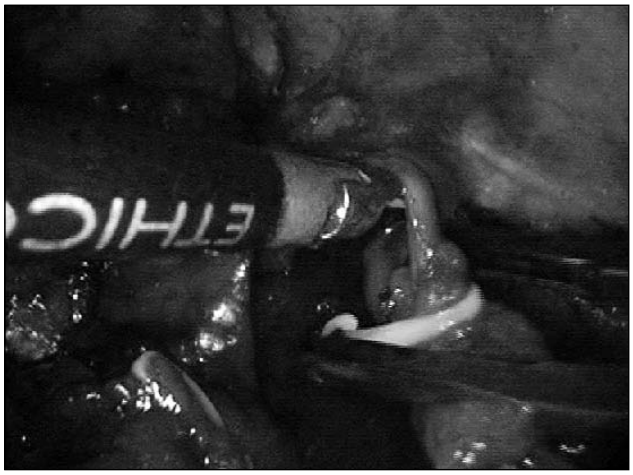


3). A second 1.0 cm incision,was made 6 cm medially to the apex of the triangle, after digital elevation of the skin throught the first incision, to place a 10mm trocar. The last 5 mm port was placed 6 cm laterally to the apex of the triangle, in an analogous manner. A 10 mm Hasson trocar was inserted in the first incision. The first port accomodates a zero degree optics. The medial port accepts the harmonic scalpel or the clip applier and the lateral port may accept the grasper, scissor or a dissection device (Fig. 4). Surgeons were positioned laterally to patient's leg and the surgery can be made ergonomically;

3. gas insuflation. The working space was insufflated with CO_2 at 15 mmHg with quick space distention, and CO_2 pressure can be kept as low as 5 mmHg during all procedure. Transilumination allows good orientation and monitorization of the progression of the dissection area towards the cavity (Fig. 4);
4. retrograde dissection and identification of anatomical limits. It is imperative that the dissection be carried out with harmonic scapel in a correct plane deep to Scarpa's fascia until the external obliquous fascia is achieved, so that all lymphatic superficial tissue can be removed (Fig. 5). The main landmarks – adductor longus muscle medially, the sartorius muscle laterally and the inguinal ligament superiorly – are well visualized (Fig. 2). At this point we identify the saphous vein medially and the spermatic cord and the external inguinal ring superomedially. The femoral nerve branches, which can be preserved, present laterally;
5. identification and dissection of the saphenous vein cranially up to fossa ovalis (Fig. 6);

Figure 5. Dissection of the correctly plane is made with harmonic scapel, maintained Scarpa´s fascia adhered to the skin to prevent ischemia.

Figure 6. Saphenous vein is ligated and transected in the vertex of the femoral triangle, to permit the dissection of lymphatic tissue proximally.



6. femoral artery identification at the femoral triangle (lateral edge of dissection limit). At this point it is recommended to open the muscular fascia in all its extension (Fig. 7);
7. distal lymphatic tissue ligation at the femoral triangle vertex. The tissue is dissected with harmonic scalpel and the final control is obtained with clips;
8. lymphatic tissue dissection reaches the femoral vessels above the femoral ring;
9. distal sapenous ligation with metallic or polymeric clips;
10. control of saphenous branches with harmonic scalpel or clips and proximal ligation of the safena vein at the femoral vein with metallic or polymeric clips (Fig. 8);
11. end of dissection, liberating the specimen after ligation of the proximal portion of the lymphatic tissue at the deep portion of the femoral channel (Fig. 9);
12. specimen removal thought the first 15 mm incision. If the specimen is larger, the incision can be enlarged, usually by 20 or 25 mm (Fig. 10);
13. sucction drainage at the 5 mm port incision;
14. suture of incisions (10-20 mm) (Fig. 11);
15. perioperative care and follow-up. Prophylatic intravenous cefalotin was done routinely. In the post operative period patients were estimulated to early ambulation and none received anticoagulants. Oral intake was started 12 hours after the procedure. Suction drain was removed when output less than 50 ml.

Figure 7. Femoral artery at the femoral triangle.

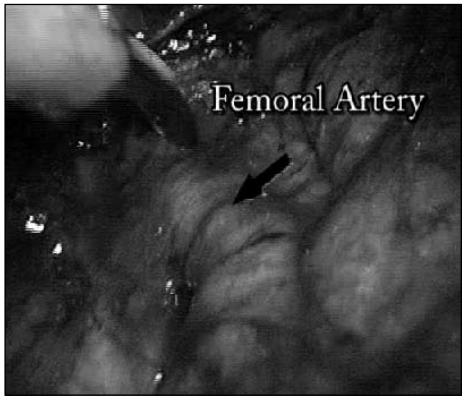


Figure 8. Dissection of saphenous insertion in femoral vein, after transection of superficial branches.

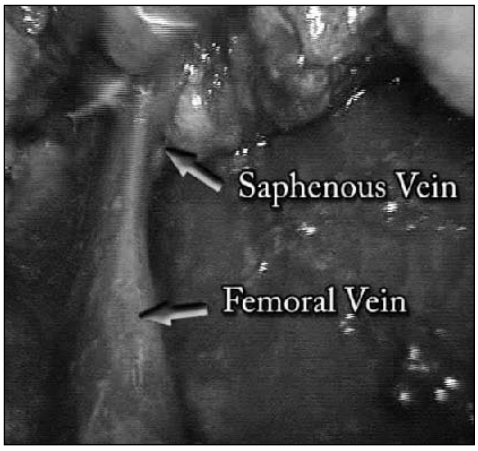


Figure 9. Dissection of femoral channel, medial to femoral vein and under the inguinal ligament, removing the Cloquet node.

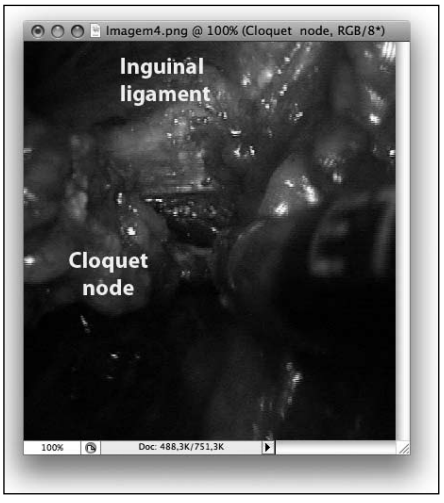


Figure 10. After completed the lymphadenectomy, the Hasson trocar is removed and the specimen is extracted through the 15 mm incision.

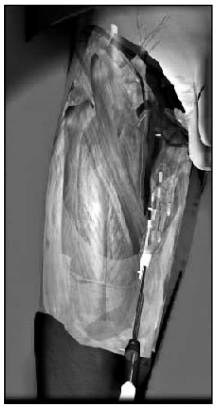
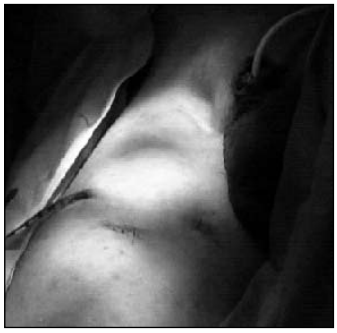


Figure 11. Continuous suction drain is positioned in the 5 mm port, and the others incisions are closed.



Conventional VEIL with saphenous vein preservation

The dissection must spare saphena and lymphnodes are ressected in 2 blocks (lateral and medial). Sometimes is possible identify major and accessory saphena and preserve both (Fig. 12). Deep dissection was performed without further problems or limitations.

Symplified VEIL (Catalona's template) and frozen section

Some authors prefere dissect only lymphonodes medial to the saphena and perform a frozen section (Fig. 13). In cases when pathologic examination were positive for malignancy an extended template are amplified. Sotelo el al showed that symplified dissection could be performed in 30 min less than radical dissection [25]. It´s important to stress that some reports derived from open surgery consider simplified dissection unreliable due to 15% of late recurrence [13].

Figure 12. Lymphadenectomy preserving magna and accessory saphenous veins.

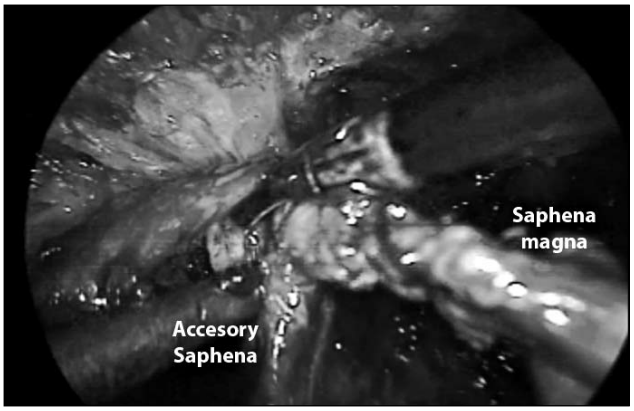
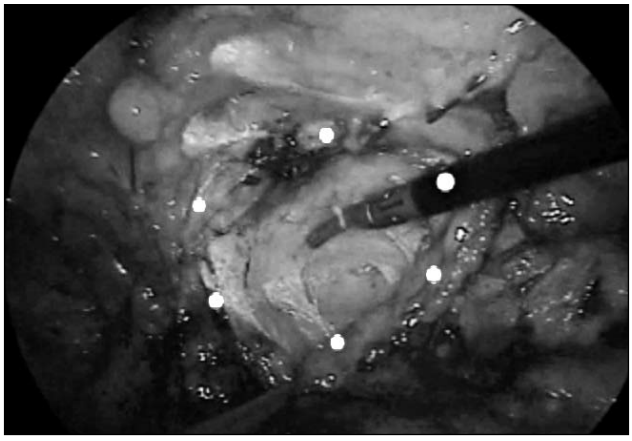


Figure 13. Resection of superficial inguinal lymphonodes, proposed by Catalona. The white circles delimited the area of dissection, medial to the saphenous vein.



Superficial VEIL

Based in the cost X benefit binomia some authors consider open superficial dissection the gold standard of care [26 27].

In the endoscopic technique this is the first step of conventional VEIL without the dissection of deep nodes of femoral channel [28]. Position of superficial lymph nodes can be localized by sonography and their projection marked with black ink to eisier ressection during surgery. Frozen section can be done as performed after simplyfied dissection [25].

Bilateral VEIL

An initial study in 5 patients suggest that all advantages of video endoscopic surgery can be obtained in this approach [23]. Mean operative time was 4.5 h. Hospital stay was 24 h (12-36 h). Mean time to drainage withdraw was 5 days (3-7 d). Mean time to recovery to normal activities was 14 days (7-18 d).

Robotic VEIL

First two dissections in 2 steps in the same patient was recently described for Josephson et al. [29]. They performed surgery with da Vinci assistance (3 ports) and 1 port for suction and clipping by the assistant. The authors reported results similar to previous publications with conventional VEIL.

The ergonomic position of surgeon and the flexibility of manuvers are the great advantages of this new option. The disadvantages include the costs and

the non availability in most centers that treat penile cancer [30].

Learning curve

Open inguinal lymphadenectomy is not a routine operation for most urologists. In primary perception VEIL seems to be a difficult technique. The working space is small but familiar to surgeons who work with the extraperitoneal endoscopic access. Conversely, the respect of open principals as mantain a good thickness of skin flap, identification of anatomical parameters and ressect all lymphatic tissue of this region seem to be achieved with few procedures for surgeon with experience in open lymphadenectomy and endoscopic techniques [31].

Operative time is greater in the learning curve compared to the the open procedure, but we believe that it will be decreased soon.

In our experience when comparing the first 10 and the last 12 procedures there was a small reduction in mean operative time (120 to 105 min), but there were no differences in complication rate. The mean number of ressected nodes are slightly highier with experience.

Comments and future directions

There are some controversial issues concerning the prophylatic inguinal lymphadenectomy in patients with penile cancer.

Some authors published data about the immediate lymphadenectomy advantages [4] while others recommend watchful waiting police and salvage surgery when the inguinal lymphonodes become clinically positive [32].

Although the survival benefits when performing lymphadenectomy in patients with impalpable lymphonodes had been demonstrated, the surgical morbidity is still high [3 5 6 32].This conventional surgery is frequently performed with a big inguinal incision and can present skin complications such as skin necrosis and wound infection. Depending the ganglionar resection extension, leg and thigh chronic lymphedema, lymphorrea and lymphocele can occur. More recent publications suggest that the application of some intraoperative and postoperative measures can partially decrease the complication rates [7].

During the last two decades, the management of penile carcinoma patients with impalpable regional lymphonodes has improved, making the procedure considerably less morbid than before. There are some reasons to explain these improvements.

Due to the fact that patient's selection has improved and surgery has been avoided in patients with low risk of lymphatic disease [2 15]. Addictionaly some authors perform a limited area of dissection with preservation of the saphenous vein [8-11]. Although their morbidity have decreased, all of this techniques did not reach the optimal oncological control.

More recently, D'Ancona e cols reported less complications with a simplified staged lymphadenectomy compared with the radical dissection. On the other hand, 5.5% of patients with negative simplified dissection had inguinal disease during the follow-up [9].

Other strategy that has been worldwide accepted is the use of lymphoscintigraphy to attempt to detect the functional sentinel lymphonode [10]. Although the excellent results in reduction of surgical morbidity, Kroogan e cols recently showed that this kind of procedure had 15% of late inguinal recurrence which can possibly compromise patient's prognosis [12]. Addictionally, high grade of standardization is necessary to obtain acceptable results.

The description about use of laparoscopic techniques for pelvic and retroperitoneal lymphadenectomies in urologic malignancies, including prostate, bladder,penile and testicular cancers, dates from 20 years ago [33].

VEIL is a procedure in your infancy. The initial propose was to offer a radical surgery with less morbidity.

Other technical variations were proved feasible and dependent of surgeon preference [24 25 28 29].

Open superficial dissection has been proposed by some groups as standard [26 27]. Endoscopic approach can reproduce open surgery with less skin complications [28].

Saphenous vein can be preserved as some authors claim that it may reduce postoperative edema [8 9 24 25].

To whom that preconize simplyfied dissection with intraoperative frozen section biopsy [9], the endoscopic technique can also be applied. Even more postoperative benefits ocurred in 30% of patients (node positive at frozen section) that underwent to extended template (radical surgery) [25 34].

Concerning the complications, actual results are encouraging suggesting that this technique has the potential to reduce post-operative morbidity. The most important advantage of VEIL seems to be a decrease in skin events.

We believe that the reduction of morbidity may be explained by the fulfilment of the following principles:

1. minimal use of eletrocautery and avoidance of mechanical retraction;

2. small incisions, allowing better preservation of the skin blood supply and lymphatic drainage;
3. incisions away from the great vessels, that make a sartorius muscle flap rotation unnecessary;
4. identification of small lymphatic vessels under magnification and their control with harmonic scalpel and control of bigger branches with clips are imperative steps to minimize lymphatic leakage and lymphocele formation.

Hypercarbia can occur but it is easily managed with hyperventilation and hyperhidration, without any clinical repercussions. Postoperative pain seemed smaller at the endoscopic surgery.

Patient subjective preferences confirm that VEIL is an attractive minimally invasive technique.

The measurement of the bigger incision in VEIL was 2.5 cm, compared to 10 cm for the open surgery. Due to small dimension of incisions, intradermic suture can be done with more aesthetic aspect.

The benefits regarding quick discharge was obtained in bilateral surgery [23 25]. The smaller drain output on the endoscopic procedure allows us to remove the drain sooner and patient can be discharged earlier [23].

The similar number of nodes removed in both sides at the same patient is an indirect sign that VEIL can be as effective as open approach. Our follow-up is still intermediate to evaluate the oncological control, but the lack of reccurence and port implants including patients with positive nodes are encouraging [23].

Some reports of experts has considered VEIL as an interesting approach [35-38].

In terms of reprodutivity preliminary results of a ongoing word wide survey identified 11 centers (6 in Brazil, 1 in Venezuela, 1 in Equador, 1 in USA, 2 in India) when VEIL was applied. The overall results concerning morbidity and oncological control seems to be similar (non published results) [23-25 29 39 40].

New fronteirs for the future include new imaging methods to localize metastasis, as nanoparticles MRI, artefacts to better endoscopic identification of nodes, techniques to reduce lymphatic events and robotic surgery. Reduction of learning curve and ergonomic issues are the most important advantages of robotic tecnology.

Conclusion

VEIL is a safe and feasible technique to patients with penile carcinoma.

Preliminary results suggest that VEIL allows a decrease in postoperative morbidity without compromising the oncological control.

Based on data avalable in the literature, VEIL has the potential to become the chosen minimally invasive procedure to prophylactic inguinal lymphadenectomy in patients with penile cancer.

New reports with more patients and a larger follow-up will be necessary to define the real value of this new technique in the modern urologic oncology armamentarium.

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Modified inguinal lymphadenectomy for penile carcinoma has no advantages

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Summary

Introduction. In 1988 Catalona reported on a modification of the classical radical lymphadenectomy for penile carcinoma; during the last decades our group has adopted it, and now the results are reported.

Methods. Between 1998 and 2006, 32 patients (58.9 ± 14 years) have been treated at our institution for penile carcinoma with penectomy and inguinal lymphadenectomy. Standard surgery was a bilateral modified lymphadenectomy as proposed by Catalona, and frozen section analysis. For those with positive superficial nodes, classical radical lymphadenectomy was performed bilaterally. Pelvic lymphadenectomy was performed if there was evidence of pelvic disease at CT scan.

Results. Mean follow-up was 31.3 months (range 3.7-84.0). In 12 men (37.5%) there was unilateral metastatic lymph node involvement, in 4 (12.5%) bilateral and in 16 men (50.0%) there was no nodal involvement. The mean number of lymph nodes excised was 15.8. One man with a negative dissection died of the disease, and one was lost to follow-up (mortality rate 6.2-12.5%). For the men with positive nodes, 4 had disease-related mortality and 3 were lost to follow-up (mortality rate 25.0-43.7%). There was no difference in overall early (18.7%) nor late (12.5%) complication rates in patients with modified compared to radical lymphadenectomy. Mortality rate was also similar (3.1%, p = 0.49).

Conclusion. In conclusion, we observed similar complication rates when performing radical or modified inguinal lymphadenectomy, but a relatively high cancer related mortality for the latter. Modified radical lymphadenectomy therefore doesn't seem to be advantageous, and our group has abandoned this procedure.

Keywords
Lymphadenectomy • Penile neoplasms • Penis • Carcinoma • Squamous cell • Lymph nodes • Postoperative complications

Introduction

Squamous cell carcinoma of the penis is characterized by primary locoregional dissemination. Lymph node status is one of the most important prognostic factors, and inguinal lymphadenectomy is both a staging and therapeutic procedure [1-4]. Clinical evaluation, as well as imaging studies or fine needle aspiration cytology are not accurate to determine the presence of lymphatic spread [1 4-6].

Lymphadenectomy plays therefore an important role in the treatment of these patients, and there is a trend to perform immediate lymphadenectomy in patients with more aggressive disease, as well as patients with

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clinically positive lymph nodes [4 7]. In 1988 Catalona reported on a modification of the classical radical groin dissection, in which the lateral and caudal margins of dissection are reduced, and the saphenous vein is preserved without the need for transposition of the sartorius muscle [8]. Since this initial study, several groups have reported their experience with this technique, demonstrating good technical results [3 9-12]. During the last decade our group has adopted this technique, and now the results are reported.

Patients and methods

Between 1998 and 2006, 81 men (58.9 ± 14 years, range 37 to 90) have been treated at our institution for squamous cell carcinoma of the penis. In all cases penectomy had been performed as treatment for primary lesion. At least a 4-week course of antibiotics was administered (cefalexin), and clinical staging (palpation of the groins, chest x-rays and in some cases computerized tomography-CT). Of these, 32 underwent inguinal lymphadenectomy for therapeutic, prophylactic or failed surveillance reasons, and were included in the study. There were a total of 64 inguinal lymphadenectomies and 1 pelvic lymphadenectomy performed. All pathological analysis of tumors were reviewed and subjected to the TNM staging system.

Prophylactic lymphadenectomy was performed in all men who did not have palpable nodes or radiographic evidence of metastatic disease at clinical presentation, but with pT2 disease or greater, or pT1 with microscopic vascular invasion, primary lesion greater than 2 cm, high-grade carcinoma or poor compliance. Therapeutic lymphadenectomy was performed in all men who had any degree of palpable inguinal nodes present despite a 6-week course of antibiotics or those who later presented with palpable nodes.

Standard surgery was a bilateral modified lymphadenectomy as proposed by Catalona [8], and frozen section analysis. For those men with histologic positive superficial nodes in one side, classical radical lymphadenectomy was performed bilaterally. Pelvic lymphadenectomy was performed if there was evidence of pelvic disease at CT scan without distant metastasis.

In all cases a suction drain was used in the inguinal region postoperatively. Ambulation was stimulated on the morning of postoperative day 1. A light pressure dressing was in place over the groin until hospital discharge. Elastic stockings were used in all men. Patients did not routinely receive subcutaneous heparin anticoagulation postoperatively. All men received perioperative antibiotics, which were maintained until the drain was removed. Inguinal drains were removed when output was consistently below 50 cc daily.

All men were followed within 10 days of discharge home. Standard follow-up was then done at 1 and 3 months, and every 3 months thereafter. Follow-up consisted of clinical exam, chest x-ray and additional exams as necessary. Survival was calculated from time of primary tumor treatment. Four men were lost to follow-up.

Statistical analysis was performed using the Statistical Package for Social Sciences software (SPSS 13.0 for Mac OS X, SPSS, Inc., Chicago, Illinois). Complications were analyzed with the Pearson chi-square test. Disease-specific survival plots were made using the Kaplan-Meier method and survival rates were analyzed for significance using the log rank test. Statistical significance was determined at $p < 0.05$.

Results

Of the 32 men who underwent penectomy and lymphadenectomy mean follow-up was 31.3 months (range 3.7 to 84.0, Table I). Patients who did not die from the disease had a mean 33.1 months of follow-up (range from 14.7 to 64.3 months). Although the majority (22 of 32) had invasive disease, there were 6 patients with pT1 disease who underwent lymphadenectomy. All patients underwent bilateral lymphadenectomy, 17 with a therapeutic intent and 15 as a prophylatic procedure. Table II lists pathological node status according to primary tumor stage, and also the lymphadenectomy initial intent (prophylatic or therapeutic).

In 12 men (37.5%) there was unilateral metastatic lymph node involvement (pN1), and in 4 (12.5%) bilateral involvement (pN2). In 16 patients (50.0%) histological exam demonstrated that there was no

Table I. Demographic and clinical data on 32 patients undergoing lymphadenectomy for penile carcinoma.

	MEAN ± SD	(RANGE)
Age (years)	58.9 ± 14.0	(37-90)
Follow up (months)	31 ± 24	(4-84)
PENILE SURGERY	**%**	**(N)**
Partial penectomy	94.0	(30)
Glansectomy	2.0	(1)
Total penectomy	2.0	(1)
PATHOLOGICAL STAGE	**%**	**(N)**
pT1	31.2	(10)
pT2	50.0	(16)
pT3	19.8	(6)
pN0	50.0	(16)
pN1	37.5	(12)
pN2	12.5	(4)

Table II. Final node pathology, T stage and intent of surgery.

	PN0	PN1	PN2	TOTAL
T stage	(n)	(n)	(n)	(n)
pT1	(9)	(0)	(1)	(10)
pT2	(3)	(10)	(3)	(16)
pT3	(4)	(2)	(0)	(6)
Surgery intent	% (n)	% (n)	% (n)	(n)
Prophylatic	56 (9)	42 (5)	25 (1)	(15)
Therapeutic	44 (7)	58 (7)	75 (3)	(17)

Table III. Early and late complications of 64 lymphadenectomies for penile cancer.

COMPLICATIONS	MODIFIED (N = 32)		RADICAL (N = 32)		
	%	(N)	%	(N)	P
Overall					
Early	21.9.	(7)	15.6	(5)	0.206
Late	12.5	(4)	12.5	(4)	0.292
Lymphocele	3.1	(1)	3.1	(1)	0.508
Wound infection	6.3	(2)	12.5	(4)	0.238
Wound necrosis/dehiscence	15.6	(5)	0	(0)	0.026
Lymphedema	21.9	(7)	25.0	(8)	0.222

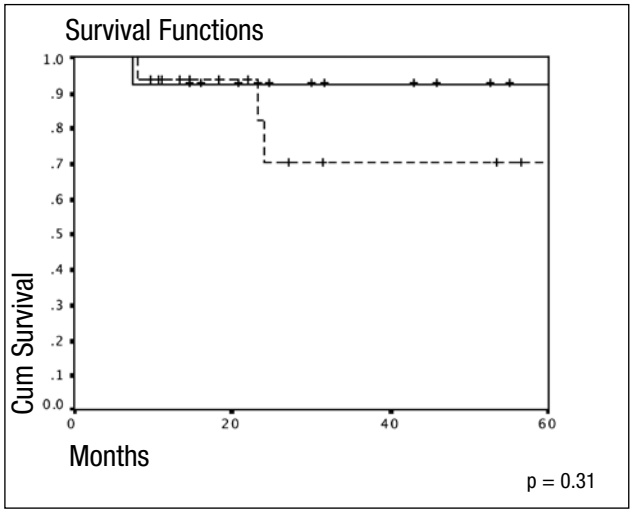
nodal involvement (pN0). The total number of lymph nodes excised ranged between 5 and 34 with an average of 15.8 nodes.

One patient died one week after radical lymphadenectomy due to meningitis (3.1%). Overall early complications occurred in 18.7% of the patients, and late complications in 12.5% (Table III). Most common complications were lymphedema (23.4%), wound infection (9.4%) and wound necrosis/dehiscence (7.8%). According to the modified Clavien system, early grade 1-2 complication occurred in 20.3% (n = 13) of procedures; No grade 3-4 complications occurred, and 3.1% (n = 1) grade 5 (death) [13].

With a follow up ranging from 4 to 64 months, one patient out of 16 (6.2%) with a negative dissection died of the disease (with local progression), and one was lost to follow-up (mortality rate 6.2 to 12.5%). For the patients with positive nodes after follow-up ranging from 8.4 to 84.0 months, 4 had disease-related mortality (25.0%) and 3 were lost to follow-up (mortality rate 25.0 to 43.7%), see Figure 1.

There was no difference in overall early nor late complication rates in patients with modified compared to radical lymph node dissections (p = 0.13 and 0.29, respectively). Mortality rate was also similar (p = 0.49).

Figure 1. Disease specific survival in patients with penile carcinoma stratified according to pathologic nodal involvement (positive - dash *vs.* negative - continuous line).



Discussion

It is well accepted that patients with clinically positive lymph nodes undergo inguinal lymphadenectomy [14]. However clinical evaluation has a low accuracy. Our study demonstrated preoperative clinical staging false-positive and false-negative rates of 41.2% and 30.8% respectively. These results were similar to previously reported, with a slight higher rate of preoperative false-negative exams [15].

For patients with microscopic metastases, early lymphadenectomy has a clear benefit in improving survival [7 16 17]. Therefore, it has been also supported by

most authors that patients at high risk for lymphatic disease should undergo a nodal evaluation [7 14].

Controversy exists regarding the best approach in such clinical situation. Sentinel lymph node dissection is still under investigation, and it is not a widely available technique [14]. And radical inguinal lymphadenectomy might be considered too aggressive as a prophylatic procedure [14]. In 1988 Catalona reported on a modification of the classical radical groin dissection, in which the lateral and caudal margins of dissection are reduced, and the saphenous vein is preserved without the need for transposition of the sartorius muscle [8]. The main advantage of this procedure would be to burden a lower complication rate [3]. Our group has adopted a selective approach, employing radical lymphadenectomy only when positive lymph nodes were detected in frozen sections during modified surgery.

Our study has some important findings. First, our data have demonstrated that complication rates were similar for patients who required radical inguinal lymphadenectomy, after a modified approach. In fact, wound dehiscence was even more common following the modified lymphadenectomy ($p = 0.01$, Table III), what doesn't seem to have an obvious reason.

Second, the main concern when treating such an aggressive malignancy should be oncological outcomes. Lopes however have demonstrated a possible oncologic unreliability with the modified inguinal lymphadenectomy [18]. Previous studies have demonstrated recurrences rates from 11-15% after modified lymphadenectomy [3 18]. We observed a cancer related mortality of 6.2-12.5% following negative bilateral modified groin dissection (with local recurrence). The failures can be explained by the fact that the lymph nodes involved in these cases might have been outside the limits of dissection of the modified lymphadenectomy. Modified lymphadenectomy should be considered a staging procedure, with 93.8-85.0% accuracy. However, squamous cell carcinoma of the penis is an aggressive malignancy, with low response to adjuvant treatment modalities when systemic disease occurs. Moreover, complication rates were similar in the modified and radical groups in the present study. Therefore, treatment should be as aggressive as the disease when there is still a major possibility to cure the patient, mainly if both surgical approaches burden similar complication rates.

In conclusion, we observed similar complication rates when performing radical or modified inguinal lymphadenectomy, but a relatively high cancer related mortality rate for the latter. Modified radical lymphadenectomy therefore doesn't seem to be an advantageous procedure. In face of these results, our group has now abandoned the modified inguinal dissection.

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Genital Human Papillomavirus in spermatozoa of young men

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Summary

Introdution. Human Papillomavirus (HPV) is a common infection and causes a wide spectrum of disease in women and in men. While in women with HPV infection and consequences have been widely studied, little is known regarding male HPV infection.
Objective. To demonstrate the presence of HPV in sperm and its associations with an alteration of sperm parameters.
Methods. This is a cross-sectional clinical study set in the Andrology and Microbiology sections at a university hospital. We enrolled 100 young males volunteers aged 18 with previous unprotected sexual intercourse. The main outcome measures were seminal parameters, sperm culture for HPV by performed PCR and FISH analysis for HPV of the sperm head. Statistical analysis was performed with a two-tailed Student's t-test.
Results. The presence of HPV infection was observed in 10 sperm samples of the 100 subjects analyzed. FISH analysis, performed on semen samples of infected and non infected subjects, resulted positive only in those 10 males previously positive to PCR analysis. Interestingly FISH analysis showed that only a part of sperm heads had a positive reaction for HPV, from a minimum of 16% to a maximum of 35% of sperm cells, with a mean percentage of 25%.
Conclusions. This study demonstrates a high prevalence of HPV infection in young adults men and among the samples of semen that had HPV, a variable percentage of infected sperm, from 16 to 35%. Furthermore the presence of HPV infection is associated with a reduction of motility.

Keywords
Human papillomavirus • Male HPV infection • Spermatozoa • Sperm parameters

Introduction

Human Papillomavirus (HPV) is one of more common sexually transmitted viral infection and causes a wide spectrum of disease. All Papillomaviruses consist of a double stranded circular DNA of around 8Kb that is made up of early and late genes. Six early genes encode for the transcription of the non-structural proteins (E1, E2, E4, E5, E6 and E7), while two late genes for the transcription of the coat proteins L1 and L2 [1]. HPV life cycle is linked to epithelial cell development and there is no viremia associated with infection. Even if most infections are transient and asymptomatic, the clinical spectrum of disease ranges from benign warts (primarily caused by low-risk HPV genotypes 6 and 11)

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to invasive malignancy (over 70% of cervical cancer is associated with the high-risk genotypes 16 and 18) [2]. Modeling estimates suggest that HPV infection is predominantly acquired in adolescence and that more than 80% of sexually active women will have acquired genital HPV by age 50. Relatively little is known about the natural history of anogenital HPV infection and disease in men. However an understanding of HPV infection in men is critical not only to reduce the risk of HPV transmission to women or to other men, but also because men, similarly to women, suffer the consequences of this infection. In fact in men anogenital warts, intraepithelial neoplasia and cancer of the reproductive and aeorodigestive tracts are commonly related to HPV infection [3 4]. The common sites of HPV detection in males are the penile shaft, glans and uretra [5], but its presence has been reported also in ductus deferens, epididymis and even in the testis [6 7]. Moreover, several reports documented the presence of HPV in the seminal fluid, but with contrasting data. Besides, several studies have shown that detection of HPV in semen samples is frequently associated with an alteration of sperm parameters as volume, viscosity, pH, count, motility and viability [8 9], however no direct relationship with male fertility has been yet demonstrated.

In this study we evaluated the prevalence of HPV infection of sperm cells and sperm parameters in a cohort of 100 young adults. Furthermore, among positive, we further evaluated single sperm cells establishing the percentage of infected spermatozoa by performing FISH analysis of the sperm head for HPV.

Materials and methods

Patients

Written informed consent was obtained from all subjects, and the study protocol was approved by the local ethics committee of our Institute. Among young males attending a project of andrological prevention, we enrolled in the study a group of students attending the last year of high school (aged 18 y) who referred unprotected sexual intercourse in the last year. A medical history including previous circumcision, smoke and sexual behaviours was obtained from each subject. No one reported previous HPV infection and physical examination did not show any specific finding of HPV. Exclusion criteria were previous history of cryptorchidism, testicular trauma or post mumps orchitis. Varicocele and seminal infections were excluded respectively by testicular doppler-ultrasound and microbiological sperm culture. All subjects collected semen for standard sperm analysis and search of HPV-DNA. All subjects (10 positive and 90 negatives) were further investigated by sperm in situ hybridization of sperm head.

Semen processing

Three semen samples collected in a 3 months period were obtained by masturbation after 3 days of sexual abstinence. After liquefaction at room temperature, semen volume, pH, sperm concentration, viability, motility and normal morphology were determined following WHO guidelines for semen analysis [10]. In each sample we performed the Sperm-Mar test (Ortho Diagnostic System, Milan, Italy) to exclude sperm antibodies. Sperm cells were then separated by Percoll gradient centrifugation and washed three times with sterile phosphate-buffered saline (PBS 1X) centrifugation and the sperm pellet used for the subsequent analyses.

Detection of HPV DNA

DNA extraction from purified sperm pellet was performed by QIAamp DNA mini kit (Qiagen, Milano, Italy). The presence of HPV DNA sequences was investigated by nested PCR using MY09/MY11 as outer primers [11] and GP5+/GP6+ as inner primers [12]. HPV-DNA positive samples were submitted to HPV genotyping by using the Linear Array HPV Genotyping test (Roche Diagnostic, Milano, Italy). The presence of HPV-DNA was also investigated by real time using the following primers and probe targeting the E7 gene: forward, 5'-ATGACTTTGCTTTTCGGGAT-3'; reverse, 5'-CTTTGCTTTTCTTCAGGACA-3'; probe, 5'-ACGGTTTGTTGTATTGCTGTTCTAA-3' for HPV-16. Real-time PCR was performed on an ABI PRISM 7900 sequence detection system (Applied Biosystems, Foster City, CA). Quantity and integrity of purified DNA was checked by quantitative real-time PCR amplification of the β-globin gene [13].

Fluorescence in situ hybridization

At least $2x10^6$ of ejaculated sperm were fixed in a methanol-acetic acid solution for at least 1 hour at -20°C. To permeabilize, samples were digested with pepsin diluted 1:25000 in pre-warmed 0,01 mol/L-1 HCl for 10 minutes at 37°C. Permeabilization of the specimens was stopped with 3-5 minute washes in PBS 1X; then samples were dehydrated in 70%, 80% and absolute ethanol for 2 minutes and finally air-dried. Samples were then overlaid with 20 µL of hybridization solution (Pan Path, Amsterdam, The Netherlands), containing biotin (BIO)-labelled HPV DNA probe (a mix of total genomes containing the conserved HPV region). Each slide was covered with

a glass coverslip and the edges were sealed with nail polish to prevent loss of the mixture during denaturation and hybridization. After a simultaneous denaturation of cellular target DNA and HPV DNA probe on a heating block for 5 minutes at 95°C, hybridization was performed by incubating the samples at 37°C overnight in a humidified chamber. Thereafter, the coverslips were carefully removed and the slides were washed in PBS 1X for 10 minutes. After 15 minutes' incubation at 37°C with the differentiation reagent (Pan Path, Amsterdam, The Netherlands) the slides were washed 3 times in PBS 1X. The negative control was processed in the same way but omitting the viral probe. The biotin-labeled HPV probe was detected by incubation with 1:200 streptavidin texas red (Vector Laboratories, Burlingame, CA) for 40 minutes at room temperature. After detection the slides were washed twice in PBS 1X/0,01% Triton and then twice in PBS 1X and mounted with a solution containing DAPI and anti-fade (BioBlue, BioView Ltd. Nes Ziona, Israel). Samples were analysed using a fluorescence microscope (Nikon, Eclipse E600) equipped with a triple band-pass filter set (FITC, TRITC, DAPI). For each slide 200 spermatozoa were analysed. Evaluation of nuclear hybridization signals was performed by 3 investigators. When nuclei were completely and homogeneously stained and when multiple small spots or single large signals were present, the sperm cells were classified as positive. The method was tested on control slides containing CaSki cells, human cervical carcinoma cell line with stably integrated and transcriptionally active HPV genomes, that served as control for the specific probe. Cells smeared on silanated glass slides were fixed with 4% paraformaldehyde in PBS for 10 min. After fixation, cells were subjected to 3-5 minute washes in PBS 1X, and then dehydrated with 5 minute ethanol washes (30%, 60% and 95%). Cell smears were then air-dried and stored at 4°C until use. For the negative controls viral probe was omitted during the hybridization procedure.

Statistical analysis

The values shown are the averages of at least three independent experiments (n = 3) performed in triplicate. Differences between data were determined by two-tailed student's t-test after acceptance of normal distribution with the Kolmogorov-Smirnov test. P values (two sided) of less than .05 were considered to be statistically significant.

Table I. Type of HPV infection and sperm parameters observed in young adults who had unprotected sexual intercourses.

ID	HPV GENOTYPE	SPERM VOLUME (ML)	PH	SPERM CONCENTRATION (MIL/ML)	SPERM COUNT (MIL)	MOTILITY (A + B) %	NORMAL MORPHOLOGY %	VIABILITY %
31	HPV-18, HPV-53, HPV-66	2.5	7.7	74	185	19	16	78
86	HPV-16, HPV-59	1.8	7.3	35	63	31	36	69
66	HPV-6	2.2	7.4	48	105	34	29	86
69	HPV-6	4.3	7.8	75	322.5	63	25	91
53	HPV-58	5.8	7.9	47	272.6	46	30	83
64	HPV-6, HPV-61	1.8	8	93	167.4	58	33	91
70	HPV-70	3.5	7	103	360.5	35	35	88
94	HPV-6, HPV-16, HPV-62	2.7	7.6	68	183.6	54	44	94
200	HPV-70	1.6	8.1	8	12.8	16	27	79
185	HPV-16	2.9	7.9	24	69.6	21	40	76
Infected (n = 10)		2.9 ± 1.6	7.7 ± 0.3	57.5 ± 30.4	174.3 ± 115.8	37.7 ± 16.8	31.5 ± 8	83.5 ± 7.9
Non infected (n = 90)		2.4 ± 1.6	7.6 ± 0.2	60.2 ± 31.0	175.8 ± 154.5	53.7 ± 18.2	33,1 ± 11.1	84.6 ± 8.6

Mil: millions; SD: standard deviation. Data from infected and non infected subjects are expressed as mean ± SD.

Results

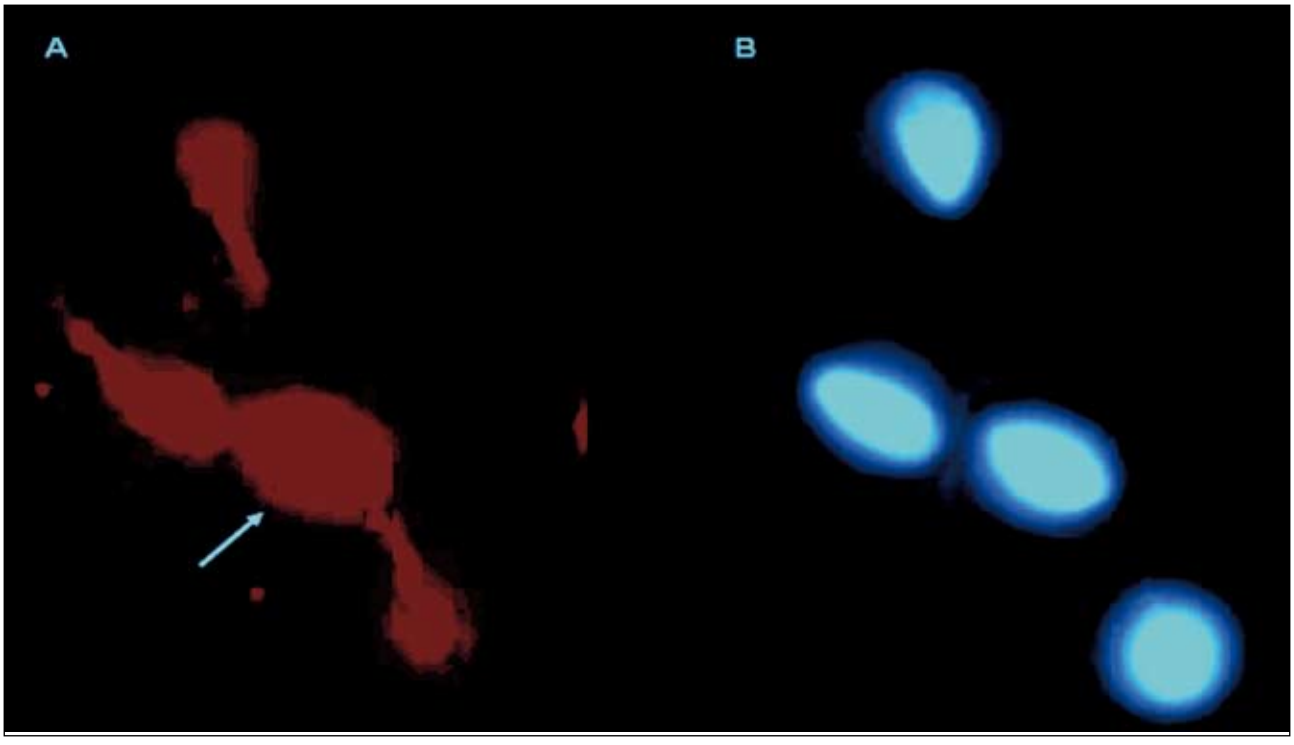
Nested PCR amplification of HPV DNA and real-time PCR for HPV-16 DNA, performed on sperm DNA of young adult males who had previous unprotected sexual intercourses resulted positive in 10 subjects (10%). The frequency of intercourse, the mean number and range of both sexual partners and age at the first sexual intercourse were not different in infected and in non infected subjects. Among infected samples a co-infection by different HPV types was observed in 4 cases. The genotype of HPV detected in these subjects and their sperm parameters are reported in table 1 as sperm parameters of non infected subjects. The reported sperm parameters are the mean of three different semen analyses. Seminal volume, pH, sperm concentration, viability and normal morphology were not different in HPV infected and in non infected sperm samples. In contrast, a significant reduction of mean sperm motility was found in the 10 semen samples resulted positive for HPV (motility a + b 53.7 ± 18.2 in HPV-negative versus 37.7 ± 16.8 in HPV-positive; $p < .05$). In particular, seven out of ten infected samples (70%) had an impaired sperm motility (motility a < 25% and a + b < 50%) whereas the result was 27 out of 90 (30%) among non infected ($p < .05$). Furthermore, we analyzed the 10 HPV infected sperm samples and 40 more HPV non-infected samples by fluorescence in situ hybridization for HPV. None of the 90 non-infected samples but all 10 DNA-HPV positive samples, showed the presence of HPV localized at sperm head (Fig. 1). Interestingly, in the latter samples only a part of analyzed sperm heads showed the hybridization for HPV. In fact, the percentage of positive sperm cells ranged from a minimum of 16% to a maximum of 35% with a mean percentage of 25% positivity.

Discussion

Despite little attention is addressed to HPV in male, recent studies have demonstrated a prevalence of 65,2% of HPV infection among men aged 18 to 70 years [2]. Moreover some authors showed the presence of HPV in semen and demonstrated its association with an alteration of sperm parameters and in particular sperm motility and pH [8 9].

Also our data demonstrated an high prevalence of HPV infection among young adult males (10%) who previous unprotected sexual intercourse. Furthermore a contemporary reduction of mean sperm motility was observed in those semen samples with presence of HPV DNA. Among our subjects 4 were positive for high-risk genotypes (HPV-16 and HPV-18) and in 3 of them there was a co-infection with

Figure 1. Fluorescence in situ hybridization performed on sperm cells from infected subjects. A) Red: HPV positive probe (texas red); arrow indicates an infected sperm. B) Blue: nuclear staining (DAPI).



other low-risk HPV types. The most frequent genotype was the low-risk HPV-6 (40%), followed by the high-risk HPV-16 (30%). These epidemiological data are in agreement with the previous published data showing HPV-6 and HPV-7 as more frequent HPV genotypes involved in male genital wart [14] and in penile squamous cell carcinoma [15] respectively.

In situ hybridization of sperm head allowed us to observe that only a part of sperm was positive for HPV, ranging from 16 to 35% of the whole sperm population. On one hand this data supports other studies demonstrating the presence of the virus in sperm cells, but on the other it doesn't clarify if HPV DNA is simply trapped in the membrane, free in the cytoplasm or even integrated into the sperm nucleus. Because of the strongly compacted nucleus of mature sperm cells, it is difficult to hypothesize a penetration of viral DNA into sperm. However a previous study [16] showed that HPV-16 and HPV-18 E6 gene-specific mRNA is expressed in human sperm, assuming that HPV genes are integrated in infected sperm cells. This finding raises the question if HPV could infect the early stages of spermatogenesis. Furthermore a previous study demonstrated that incubation with HPV is able to transfer viral DNA into sperm and that infected cells can deliver exogenous DNA to the cumulus cells surrounding ovulated oocytes at the time of fertilization [17 18].

In conclusion this study demonstrates the presence of HPV in sperm and its association with an alteration of sperm motility. These observations could suggest screening for HPV all patients affected by idiopathic astenozoospermia, after exclusion of the known causes of this anomaly and in particular in asthenozoospermic patients candidate to assisted reproduction techniques (ART) or to sperm banking. Anyway more studies are desirable to understand the clearance of HPV infection in men, toestablish the precise localization of the virus in sperm and to clarify its possible implication in male infertility.

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Diabetes, oxidative stress and its impact on male fertility

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Summary

Objective. In male infertile patients with diabetes, there are growing evidences indicating important physiopathological connections between the mechanisms of oxidative damage in terms of rate of fragmentation of the nuclear DNA, deletions of the mithocondrial DNA, imbalance of the oxidative balance, increased levels of enzymatic glication products in testicular and epididimary region, and besides on the seminal plasma. Taken the above oxidative damage as secondary to a putative radical oxygen species hyperproduction, aim of this study was to directly assess the radical oxygen species production, in basal and after fMLP-addition (to evaluate maximal leukocyte response), in infertile, diabetic patients. To verify a relatively defective functional of radical oxygen species production, the pattern of radical oxygen species response in diabetic patients was compared to that found in two controls groups. Sperm conventional parameters, including seminal leukocytes concentration were also evaluated in patients and controls groups.

Material and methods. In the present observational study, 18 selected, infertile patients with diabetes (mean age 36 years, range 28- 44 years) underwent semen analysis for assessment of radical oxygen species, sperm parameters (density, motility, morphology, and seminal leukocyte concentration). The results obtained were compared with the data obtained from two control groups (analysis among groups), including a group of 28 patients with male accessory gland infection and a group of 16 healthy, volunteers subjects.

Results. The mean values of semen parameters of the infertile patients with diabetes were significantly different from those found in both control groups, being together better than those of the male accessory gland infections control group but worse than those of the healthy subjects. In addition, the infertile men with diabetes had higher radical oxygen species chemiluminescent signals in all conditions (baseline and fMLP-stimulated) than those registered in the healthy subjects but lower than those found in male accessory gland infections group.

Conclusions. The infertile patients with diabetes studied characterized itself differing from the group with male accessory gland infections because the low leukocyte response in part, likely explained through an immuno-pathogenetic picture (defective macrophagic response) conditioned by the same basic disease. Despite in diabetic patients, the sperm oxidative stress seem less than that observed in presence of male accessory gland infections, this can likely explain their impaired sperm data.

Keywords
Diabetes • Male accessory gland infections • Male fertility • Reactive oxygen species • Spermiogram

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Introduction

The pathogenetic role of the oxidative stress originated by a seminal white blood cells (WBC) overproduction and/or iuxta-sperm of radical oxygen species (ROS) in the determining of possible spermatic alterations is known by a long time [1] and perhaps in the future assessment of oxidative stress will be introduced into routine provided to find agreement on methods [2]. The fields that have better explored such role have moved with time from the area of the idiopathic male infertility to the infertility from excretory causes post-infectious/inflammatory of the sexual accessory glands (Male Accessory Gland Infections, MAGI), witnessed also by the scientific contribution of our group [3-7]. Recently, a new clinical model of radical pathology is represented by the diabetic disease evolved in not conventional chronic, long-standing complications, as male infertility. In such care, different authors show important physiopathological connections between the diabetic disease and the male reproductive damage expressed as rate of fragmentation of the nuclear DNA, deletions of the mithocondrial DNA, imbalance of the oxidative balance, increased levels of enzymatic glication products in testicular and epidydimal region, and besides on the seminal plasma [8-10]. In addition, some authors have recently, demonstrated that experimental induction of diabetes in animal models using chemical diabetogens induced an impair testicular function progressively leading to decreased fertility [11 12]. A ROS-leukocyte related overproduction is influenced by extension to more sexual accessory glands [3] and it is showing the contribution of different subpopulations ROS producing (neutrophils and monocytes) and their level of operational efficiency [13]. Whereas, moreover, that the persistence of complicated urinary tract infection in diabetic men, poorly responsive to short-term antibiotic therapy [14], to be interpreted not always as cystitis, at least as prostatitis, in the absence of appropriate characterization as MAGI, recognizes different pathogenetic mechanisms (inhibition of phagocytosis, reduced secretion of urinary cytokine, decreased cell-mediated immunity)[15], we thought it was appropriate in a selected group of diabetic patients, with infertility, compare their patterns of spermatic ROS concentration with that of patients with proven MAGI.

Materials and methods

In the present observational study, 18 consecutive, selected diabetic patients with primary infertility were enrolled. All underwent semen analysis for assessment of sperm conventional parameters (density, motility, morphology), as well as seminal ROS production, and semen WBC concentration, performed according to standard conventional methods and techniques [16] and previous work of our group. The results obtained were related to the levels of glicosilated hemoglobin (HbA_{1c}) and on the duration in years of disease (intra-group analysis) and compared with the data obtained from two matched-group (analysis among groups). In particular, the concentration of semen WBC was determined through the morphological identification using the conventional immunohistochemical coloration. A part of the same sample was examined in order to determine the production of ROS-WBC related (ROS-WBC). Briefly, the sperm preparation was set in double, through separation on discontinuous gradient (45/90%) of Percoll and the assessment of the production of basal ROS and (f-MLP)-stimulated; (formil-leucil-fenil-alanina/Sigma Chemicals Co., ST. Louis, MO, USA) it was done on a quotation of 400 μl of cells in suspension, derived both from the sediment (fraction 90%) and from the interface 45/90% of Percoll as previously reported. The misuration of ROS was determined, adjusting the final concentration to 2.5×10^6 spermatozoa/ml in order to reduce the number of leucocytes in the percentage (fraction 90%; fraction 45%) otherwise responsible of an "overflow" reading signal in the chemioluminescence. To reduce to the minimum the methodological errors, all the spermiogram were performed by the same operator in a random way.

Statistical analysis

In this study we compared the values coming from a parametric test with those of a not parametric test, analyzing the discrepancies. Parametric Test: Student's test for multiple comparison without correction of Bonferroni - Test not parametric: Mann-Whitney rank-sum Test.

The software SPSS 9.0 for Windows was used for statistical evaluation. A statistically significant difference was accepted when the p value was lower than 0.05.

Results

The group of diabetic patients had an average aged of 36 years (ranged from 28 to 44 years old). Of them, 15/18 (83.3%) had diabetes mellitus diagnosis (DM) type 2; 2/18 had DM type 1 (11.1%); 1/18 (5.6%) had LADA (Autoimmune Diabetes of Adults).

The matched-groups included 44 not diabetic subjects aged between 20 and 43 years old (average

age 38 years old): furthermore, they were distinguished in 2 different categories:

a. *group of patients with MAGI,* constituted by 28 patients aged between 20 and 46 years old (average age 36 years old);
b. *group of the healthy, fertile subjects,* constituted by 16 subjects aged comprised between 23 and 41 years old (average age 38 years old).

Patients with DM showed spermatic density significantly lower than in the control group ($p < 0.05$). The density of MAGI patients was significantly lower than both the control group that patients with DM ($p < 0.05$). The percentage of sperm with progressive motility was found to be similar in patients with DM and MAGI, but significantly lower than in the control group ($p < 0.05$). The percentage of sperm with normal form in patients with DM was significantly lower than the group of control ($p < 0.05$). Patients with MAGI were significantly lower than patients with DM ($p < 0.05$). Patients with DM showed leucocytes concentration similar to that of the control group. Instead, patients with MAGI showed significantly higher leukocytes concentration compared to the other two study groups (Table I).

The diabetic patients showed mean values of production of ROS significatively higher in comparison with the subjects fertile and healthy both in the fraction of Percoll at 45% and at 90%, and in the basal conditions after adding of fMLP (response ROS-leucocitary maximal) (Table II).

Furthermore, inside the group of diabetic patients, the sub-group of diabetic patients with better glicometabolic compensation ($HbA_{1c} < 7\%$) showed a sperm rate of production of ROS significantly lower than that found in the subjects of the sub-group with lack of balance of moderate degree (HbA_{1c} 7-10%) and severe ($HbA_{1c} > 10\%$) both in the fraction of Percoll at 45% and at 90%, and in basal conditions after adding of fMLP. The only statistic discrepancy emerges from the comparison between the sub-group with values of HbA_{1c} comprised between 7 and 10% and the sub-group with values of $HbA_{1c} > 10\%$; in terms of lack of statistic coherence between the parametric test (significative difference) and the not parametric test (not significative difference) in the fraction of Percoll at 90% after adding of f-MLP (Table III).

In function of the duration of the diabetic disease, the sub-group of diabetic patients with duration of disease < 5 years always, in all Percoll fractions and conditions, showed mean values rate of ROS significantly lower than those found in the longer-standing matched-subgroup (duration of diabetes > 10 years) diabetic patients (Table III). The only statistic discrepancies emerge from the comparison between the sub-group with duration of disease comprised between 5 and 10 and the group with duration > 10

Table I. Conventional spermatic parameters in the diabetic patients and in the matched-groups. The values were expressed as mean + SEM.

DENSITY (MIL/ML)			NORMAL FORMS (%)		
DIABETES	**MAGI**	**CTRL GROUP**	**DIABETES**	**MAGI**	**CTRL GROUP**
33,4 ± 6.2	14,7 ± 2,2	48,4 ± 7,3	23,5 ± 2,3*	14,0 ± 1,7	29,7 ± 1,3

PROGRESSIVE MOTILITY (%)			LEUKOCYTE (MIL/ML)		
DIABETES	**MAGI**	**CTRL GROUP**	**DIABETES**	**MAGI**	**CTRL GROUP**
16,0 ± 1,8	13,0 ± 1,7	32,6 ± 2,1	0,9 ± 0,1	2,0 ± 0,1	0,6 ± 0,1

Table II. Production of sperm ROS generated in cell fractions after discontinous Percoll gradients in the diabetic patients and in the matched-groups (MAGI, and healthy fertile subjects). The values (photons, cmp x 1000) were expressed as mean + SEM.

PERCOLL GRADIENT (EXPRESSION OF ROS CHEMILUMINESCENCE)	DIABETIC PATIENTS (N = 18)	GROUP WITH MAGI (N= 28)	GROUP OF HEALTHY, FERTILE SUBJECTS (N = 16)
45% (baseline)	252,00 ± 28,70*	502,37 ± 63,99	79,27 ± 11,70
45% (f-MLP- stimulated)	373,18 ± 36,68*	711,74 ± 49,49	94,93 ± 12,79
90% (baseline)	21,29 ± 3,32*	44,04 ± 2,79	10,60 ± 1,44
90% (f-MLP- stimulated)	39,00 ± 2,91*	67,70 ± 2,79	23,13 ± 2,78

* $p < 0.05$ *vs.* healthy, fertile group.

Table III. Sperm ROS production registered in the diabetic group, in relation to levels of glico-metabolic compensation.

PERCOLL GRADIENT (EXPRESSION OF ROS CHEMILUMINESCENCE)	HbA_{1C} < 7% (N = 6)	HbA_{1C} 7-10% (N = 6)	HbA_{1C} > 10% (N = 6)
45% (baseline)	130,71 ± 25,10*	289,00 ± 10,68	368,83 ± 14,06
45% (f-MLP- stimulated)	218,43 ± 28,00*	413,50 ± 18,34	526,83 ± 20,90
90% (baseline)	8,57 ± 1,17*	20,00 ± 2,00	37,00 ± 3,00
90% (f-MLP- stimulated)	27,00 ± 2,73*	42,00 ± 1,47	51,00 ± 1,37

* $p < 0.05$ *vs.* group diabetics with $HbA_{1c} > 10\%$.

years; in terms of missed statistic coherence between the parametric test (significative difference) and the not parametric test (not significative difference) in the fraction of Percoll at 45% after adding of f-MLP; while in the fraction of Percoll at 90% after adding of f-MLP the same consideration regards the comparison between the group with duration of disease inferior to 5 years and more than 10 (Table IV).

Discussion and conclusions

Growing evidences indicating the impaired effects of oxidative sperm on reproductive function, mainly derived by an indirect approach of lesion between ROS production and varying experimental, cellular substrate (peroxidative damage to the sperm plasma membrane and DNA damage to the mitochondrial and nuclear genome). On the other hand considering that in DM patients, urinary tract infections have mainly complicated infections and are due to impaired phagocytosis inhibition, low secretion of urinary pro-oxidative cytokines, and defective cell-mediated immunity, a correct approach exploring sperm stress oxidative in DM patients may start by an initial direct analysis of ROS production in basal and stimulated-condition. This moved the our great concern for this research area. The diabetic group characterized itself differing from the group affected by MAGI because a lower WBC-mediated ROS production, mainly expressed in the 45% Percoll fraction (and particularly, maximal WBC response induced by fMLP addition). This ROS production pattern compared that found in the both matched-groups, could likely be attributable to an immunoreactivity picture (defective macrophagic response) conditioned by the same basic disease. On the other hand, the ROS hyperproduction indicative of iuxtasperm-originated oxidative stress (mainly expressed in the 90% Percoll fraction, which represents a WBC-poor fraction) of the diabetic patients observed in the course of the study place itself on an intermediate level between the one of the healthy subjects of control and the group with MAGI. In particular, with a basal production of ROS substantially overlapped in presence of infections of the accessory sex glands we recorded a higher production of ROS after induction with f-MLP, indicating the different sperm origin of ROS in the diabetics and/or other leukocyte (lymphocytes and monocytes) source not noticeable with routine coloration. Therefore it's assumable that the model of radical sperm pathology characterizing the diabetics could recognize 2 possible mechanisms of primer: a) sperm production; b) susceptibility to the chronicisation of the inflammatory response. It will be interesting to characterize in future the eventual fragmentation of the spermatic DNA through cytofluorimetric method and/or the correlation between the altered vesicular compliance and the cytochemical pro-inflammatory cataloguing.

Table IV. Production of sperm ROS production: differences for duration of diabetic disease.

PERCOLL GRADIENT (EXPRESSION OF ROS CHEMILUMINESCENCE)	YEARS OF DISEASE < 5 (N. 8)	YEARS OF DISEASE 5-10 (N. 5)	YEARS OF DISEASE > 10 (N. 5)
45% (baseline)	117,17 ± 25,00*	286,86 ± 15,63	389,75 ± 7,12
45% (f-MLP- stimulated)	204,83 ± 28,96*	419,00 ± 26,44	545,50 ± 25,53
90% (baseline)	7,83 ± 1,08*	21,71 ± 2,49	40,75 ± 2,84
90% (f-MLP- stimulated)	24,80 ± 3,37*	42,29 ± 2,00	52,75 ± 1,25

* $p < 0.05$ *vs.* sub-group with diabetes from more than 10 years.

Our data encourage future studies to establish the impact of antioxidants compound in the specific management of diabetic infertile patients.

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Predictive factors of better improvement in semen quality after sclerotization of varicocele: preliminary report

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Summary

Introduction. Controversial data on effectiveness of varicocele correction are available, and still clear predictive factors of better semen quality improvement are lacking. To determine whether age of patients, clinical classification and colorDoppler classification are related to a different semen quality improvement after sclerotization of varicocele.

Material and methods. 113 patients with left unilateral varicocele were selected between 2002 and 2007, as they met the criteria of low sperm density, no endocrinological failures and no recidivation of varicocele after correction. All patients underwent retrograde percutaneous sclerotization of varicocele or, if not possible, anterograde sclerotization. All patients underwent physical examination, FSH measurement, seminal analysis (sperm density, motility and percent of regular-morphology sperms), scrotal ultrasonography and colorDoppler scrotal evaluation. At least 3 months postoperative, they were assessed with the same protocol.

Results. Mean age was 32,2 yr. We found improvement in seminal parameters among the whole population (sperm density: +92,4% percent of mobile cells: +42,2%, percent of normal cells: +21,7%; $p < 0,001$). We found no significance in differences among semen quality improvement of patients of different ages (sperm density variation among patients 18-29 yrs: 14,7; 30-39: 10,2; > 39: 20,3; $p > 0,2$). We found no significance in differences among semen quality improvement of patients with subclinical *vs.* clinical varicocele. Significant evidence of higher improvement in semen quality parameters have been found among patients with basal renal reflow at preoperative evaluation *vs.* patients without basal renal reflow (sperm density: +139% *vs.* +53%, $p = 0,006$).

Conclusions. Treatment of varicocele results in improvement of seminal quality among all patients. Age is not related to a different outcome. The presence of a basal renal preoperative reflow is associated with higher improvement in semen quality parameters after sclerotization.

Keywords
Color-Doppler ultrasonography • Infertility • Sclerotization • Seminal parameters • Varicocele

Introduction

Varicocele is an extremely common entity among male population: it is found in 35%-40% of men with primary and in 75%-80% of men

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with secondary infertility [1 2]. It is considered the most frequently encountered surgically correctable cause of male infertility: Mac Leod first described the triad of oligospermia, decreased sperm motility and increased percentage of immature sperm cells, which are the typical seminal characteristics of infertile men with varicocele [3]. As varicocele correction often results in improvement in semen quality [1], several studies related varicocele size with the effectiveness of surgical varicocele correction [4 5]. However the data remain controversial to support or disprove the contention that repair of small and subclinical varicoceles improves spermatogenesis [6], and even the role of patient's age on the effectiveness of varicocele's treatment is not completely clear. Therefore our prospective study's goal was to determine predictive factors of a better improvement in seminal parameters after sclerotization of varicoceles. We studied patients' age to determine whether age of patients was related to different results in semen quality improvement and varicocele's grade to verify the role of clinical classification and the role of color Doppler ultrasound (CDU) classification in semen quality improvement after sclerotization.

Materials and methods

Patients

Between 2002 and 2007, 161 patients presented to our clinic with an unilateral left varicocele, because of either infertility at least 1 year in duration or scrotal pain. Patient age ranged from 18 to 44 years (mean 32,2). All patients underwent a complete history, physical examination in a warm room, assessment of FSH value, semen analysis and scrotal ultrasonography with color-flow Doppler examination. The subjects underwent surgical correction of their varicocele, with a retrograde percutaneous sclerotization technique, or, when not possible, anterograde sclerotization technique. At least 3 months after surgery, semen analysis and scrotal CDU have been performed. One hundred thirteen patients met the following criteria: low sperm concentration, no endocrinological abnormalities and no varicocele recidivation after sclerotization, and were included into this prospective study. The subjects have been divided into 5 groups, based on their CDU varicocele classification grade as described by Sarteschi [7].
Demographic and baseline characteristics were similar between the five groups (Table I).

Ultrasound technique

High resolution ultrasound studies were performed by direct contact with a *Esaote* AU5, with 7,5 MHz transducer. Patients, in a warm room, were first scanned during quite respiration and during Valsalva maneuver while supine, then while standing. Serial scans of each sac were performed both in transverse and longitudinal plane in order to evaluate testicles, epididims and the veins of the pampiniformis plexus. The CDU showed the presence or absence of venous reflow during base conditions or Valsalva maneuver. The varicocele classification based on the results of the CDU, as described by Sarteschi, has been used in this study because it seems to represent the more rational pathophysiological approach to varicocele classification.

1st grade: no varicose veins evident in B-Mode Ultrasonography; CDU, with transducer put on the scrotal root, shows the presence of venous reflow during Valsalva, longer than 2 seconds.

2nd grade: B-Mode evidentiate small varicose veins, reaching the superior pole of the testicle. These veins increase their diameter during Valsalva; CDU shows the presence of reverse venous flow during valsalva in the region above the testicle.

3rd grade: B-Mode Ultrasonography evidentiate varicose veins surrounding the testicle, that increase their diameter during Valsalva;

Table I. Population characteristics and mean seminal parameters before sclerotization of varicocele.

GRADE	MEAN AGE (YRS)	# OF CASES	CONC[A]	MOTIL[B]	MORPH[C]
1	32,2	7	9,13	24,7	32,9
2	30,9	21	12,6	23,5	28,1
3	32	38	17,9	18,4	35,3
4	32,4	34	16,1	23	2707
5	31,5	13	9,8	17	33,1
TOT.	32,2	113	14,9	20,9	31,8

[a] sperm concentration: millions/mL; [b] percentage of sperms with A+B motility (see text); [c] percentage of sperms with regular morphology.

CDU shows the presence of peritesticular venous reflow only during Valsalva.

4th grade: B-Mode evidentiate varicose veins during quite respiration while supine, that increase their size during Valsalva or while standing; CDU shows the presence of basal venous reflow in quite conditions, that increases its strength during Valsalva or while standing.

5th grade: varicose veins are evident in quite conditions, and don't modify during Valsalva; CDU shows continuous venous reflow that don't undergoes any modification with Valsalva maneuver or while standing.

Semen analysis

Specimens were obtained by masturbation after at least 3 days of abstinence. The specimens were valuated within 1 hour from collection for the following parameters: sperm concentration (normal range: > 20millions/mL), percentage of sperms with A + B motility (A: speed linear motility, B: slow linear motility, C: motility *in situ*, D: no motility at all), percentage of morphologically typical sperms.

Surgical technique

All patients underwent sclerotization of their left spermatic vein, using the transfemoral retrograde percutaneous approach or, if not possible, the anterograde approach during the same session. In the retrograde sclerotization the femoral vein is entered below the inguinal ligament using the standard Seldinger technique. A 6-Fr Cobra3 femoral visceral catheter is commonly used to catheterize selectively the renal vein. Renal phlebography is carried out by injection of 20 ml of water-soluble contrast enema under Valsalva manoeuvre. The catheter is often changed for another, endhole one, for selective catheterization of the left spermatic vein. After superselective catheterization of the spermatic vein, a guide wire is introduced deeply into the vein and the first catheter is replaced by a smaller one previously curved for this purpose. This catheter permits very distal catheterization. Superselective angiography shows every possible collateral circle and the possible presence of more than 1 spermatic vein. Sclerotization technique is performed injecting 2-4 mL of sodium tetradecyl sulfate 3% during a modest Valsalva maneuver, at least 10 seconds long. Should there be bulky veins, the operation is repeated at a higher lombar level. After this procedure, a control venography shows the flow coming to a stop.

Statistical analysis

Statistical analyses were performed with SPSS 15.0 software package. The significativities of differences between preoperative and postoperative means values for each group have been valued with the non parametric Wilcoxon signed rank test; Means variations between different groups has been valuated with U-Mann-Whitney test. The research for a significative linear correlation between age and the parameters completed the statistical analysis. Probability values < 0,05 were considered significant.

Results

The seminal parameters analyzed showed a significant increase among our population: sperm concentration increased from 14,9 millions per cc preoperatively to 28,7 (+92.4%, $p < 0,001$); percent motility increased from 21% preoperatively to 30,9% postoperatively (+42,2%, $p < 0,001$); sperms with a regular morphology increased from 31,8% to 38,7% (+21,7%, $p < 0,001$). Eighty-two percent of our patients underwent a postoperative increase of sperm concentration, 73% underwent a postoperative increase of sperm motility, and 58% of our patients underwent an increase in normal morphology. Sixty-seven percent of our patients showed a postoperative increase in both concentration and motility.

At first patients have been divided into 5 groups, based on the grade of their varicocele as described by Sarteschi's classification. Among *1st group*: sperm concentration varied from 9,16 millions per cc to 28,6 ($p = 0,018$), motility and percentage of morphologically regular cells did not significantly increase. Among *2nd group*: sperm concentration varied from 12,6 to 21,6 millions per cc (+72%, $p = 0,003$). Motility increased from 23,4% to 35,2% (+50%, $p = 0,003$). Percentage of morphologically regular cells increased not significantly. Among *3rd group*: concentration varied from 17,6 to 25,6 millions per cc (+50%, $p < 0,001$); motility increased from 18,4% to 24,1% (+31,6%, $p = 0,006$); percentage of morphologically regular cells increased from 35,3% to 41,2% (+16,9%; $p = 0,001$). Among *4th group*: concentration varied from 16,1 to 36 millions per cc (+123%, $p < 0,001$); motility increased from 23% to 34,2% (+49%, $p < 0,001$); percentage of morphologically regular cells increased from 27,7% to 37,6% (+36,3%; $p = 0,031$). Among *5th group*: concentration varied from 9,8 to 30,1 millions per cc (+209%, $p = 0,01$); motility increased from 17% to 28,5% (+68%, $p = 0,028$); percentage of morphologically regular showed no significant increase (Tables II-IV).

Table II. Comparison of sperm concentration in patients before and after sclerotization, and analysis of variation's significance.

GRADE	CONC PRE	CONC POST	CONC VAR	% VAR	P VALUE
1	9,13	28,64	19,51	213,7%	0,018
2	12,59	21,62	9,03	71,7%	0,003
3	17,59	25,67	8,08	45,9%	< 0,001
4	16,14	35,95	19,81	122,7%	< 0,001
5	9,75	30,12	20,37	208,9%	0,01
Tot	14,9	28,7	13,8	92,6%	< 0,001

Table III. Comparison of percentages of mobile sperms (type A + B, see text) in patients before and after sclerotization and analysis of variation's significance.

GRADE	MOTIL PRE	MOTIL POST	MOTIL VAR	%	P VALUE
1	24,71	41,86	17,15	69,4%	*0,062*
2	23,48	35,19	11,71	49,9%	0,003
3	18,38	24,18	5,8	31,6%	0,006
4	22,99	34,21	11,22	48,8%	< 0,001
5	16,95	28,46	11,51	67,9%	0,028
Tot	20,9	30,8	9,9	47%	< 0,001

Table IV. Comparison of percentages of sperms with normal morphology before and after sclerotization and analysis of variation's significance.

GRADE	MORPH PRE	MORPH POST	MORPH VAR	%	P VALUE
1	32,86	41,29	8,43	25,7%	0,063
2	28,11	32,31	4,2	14,9%	0,068
3	35,25	41,22	5,97	16,9%	0,001
4	27,7	37,76	10,06	36,3%	0,031
5	33,08	38,7	5,62	17,0%	0,44
Tot	31,8	38,7	6,9	21,6%	< 0,001

Table V. Variations of sperm concentration and percentage of mobile sperms (A + B, see text) preoperatively and postoperatively, referred to patients' age at intervention.

AGE	#	GRADE	CONC PRE	CONC POST	CONC VAR	MOT PRE	MOT POST	MOT VAR
18-29	37	3	16,6	31,3	14,7	25,6	37	11,4
30-39	52	3	14	24,2	10,2	17,5	28	10,5
> 39	24	4	14,1	34,4	20,3	31,1	28,8	-2,3

Patients have been then divided into 3 groups, based on their age at operation time: 37 between 18 an 39 years, 52 between 30 and 39, and 24 patients more than 39 years old. A comparative analysis between the preoperative and postoperative values of the seminal parameters has been performed, but showed no significant evidence of differences between mean's improvements of these groups (sperm density variation among patients 18-29 yrs: 14,7; 30-39: 10,2; > 39: 20,3; $p > 0,2$; Tables IV, V) The research of a linear regression between each parameter and the age at operation time confirmed the absence of an evident significant correlation between age and both preoperative and postoperative values (Table VI).

Patients have been divided into 2 groups, based on the clinical evidence of their varicoceles: grade 1+2

Table VI. Analysis of significance in parameters variations between patients of different ages.

GROUPS	CONC VAR	P VALUE	MOT VAR	P VALUE
18-29 vs. 30-39	-4,5	0,2	-0,9	0,7
30-39 vs. > 39	10,1	0,049	-12,8	0,4
18-29 vs. > 39	5,6	0,37	-13,7	0,2

formed subclinical group (28 patients); grade 3+4+5 formed clinical group (85 patients). Among patients with a subclinical varicocele, sperm concentration varied from 11,7 to 23,4 millions per cc (+99%, $p < 0,001$); motility increased from 23,8% to 36,9% (+55%, $p < 0,001$); percentage of morphologically regular cells increased from 29,8% to 35,5% (+11%; $p = 0,006$). Among patients with clinical evidence of varicocele, sperm concentration varied from 15,6 to 30,4 millions per cc (+91%, $p < 0,001$); motility increased from 20% to 30% (+45%, $p < 0,001$); percentage of morphologically regular cells increased from 32,4% to 39,7% (+22%; $p = 0,006$) (Fig. 1). There is no significant difference between values' improvement between these groups (p value varied from 0,1 to 0,8).

Patients have been then divided into 2 more groups, based on the absence or presence of basal venous reflow at CDU investigation: grade 1+2+3 formed the no basal reflow-group (66 patients); grade 4+5 formed the basal venous reflow-group (47 patients). Among patients with a varicocele with no basal venous reflow, sperm concentration varied from 15,3 to 24,7 millions per cc (+61%, $p < 0,001$); motility increased from 20,7% to 29,6% (+43%, $p < 0,001$); percentage of morphologically regular cells increased from 33% to 39% (+10%; $p < 0,001$). Among patients with ColorDoppler evidence of venous spermatic reflow, sperm concentration varied from 14,3 to 34,3 millions per cc (+139%, $p < 0,001$); motility increased from 21% to 32,7% (+53%, $p < 0,001$); percentage of morphologically regular cells increased from 24,4% to 38,1%% (+29,3%; $p = 0,006$). The difference between sperm concentration improvement among the two groups showed statistical significance ($p = 0,02$; Fig. 2).

Discussion

In our study we found a significant improvement in semen paramenters after sclerotization of varicocele; no correlation between patient's age and improvement in semen quality; no significant differences in semen quality improvement between patients with clinical and subclinical varicoceles and a significant better improvement in semen quality parameters among those patients who presented basal venous reflow at CDU preoperative investigation.

The association of varicocele with infertility has been recognized for more than 50 years [8]: varicocele causes a duration-dependent decline in semen analysis parameters due to higher scrotal temperature, reflow of toxic metabolites, local hypoxy and lack of nutrient factors [9-12]. Several studies tried to determine the progression of seminal parameters after surgical correction of varicocele, but the results are yet discussed. Although most of them show a significant postoperative improvement of seminal parameters in 55-75% of treated patients [5 13 14], recently some authors performed a meta-analysis where every possible beneficial effect of varicocele treatment is

Figure 1. Comparison between variations in seminal parameters among patients with subclinical (grade 1 + 2) and clinical (grade 3 + 4+ 5) varicocele.

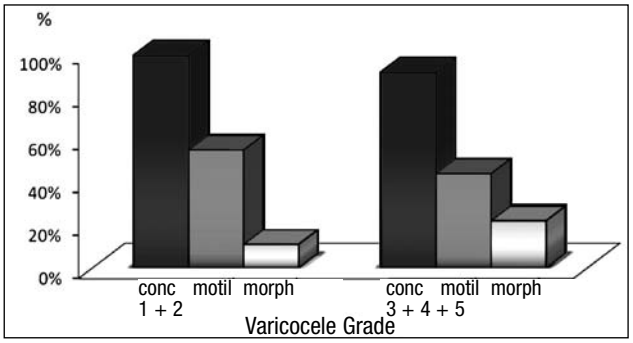

Figure 2. Comparison between variations in seminal parameters among patients with no evidence of preoperative basal CDU reflow (grade 1 + 2 + 3) and patients with evident basal CDU reflow (grade 4 + 5).

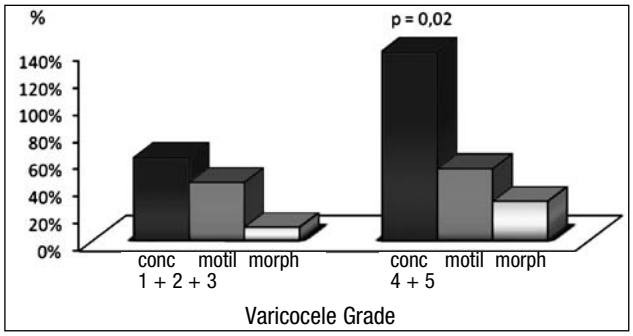

Table VII. Analysis of significance in linear regression obtained assuming each single preoperative and postoperative sperm parameter and its variation as the dependent variable and patients' age as the independent variable.

DEPENDENT VARIABLE	LINEAR REGRESSION	P VALUE
Conc pre	-0,13	0,5
Conc post	0,156	0,7
Conc variation	0,285	0,4
Mot pre	-0,35	0,166
Mot post	-0,555	0,05
Mot variation	-0,2	0,4
Morph pre	-0,5	0,19
Morph post	-0,3	0,12
Morph variation	-0,03	0,9

denied: this Cochrane Libraries Review failed to offer evidence that treatment of a varicocele in men does improve couple's spontaneous pregnancy chances, and the authors suggested that as long as it still unclear whether it is true or not that a varicocele is "nature's attempt to heal a diseased testis rather than afflict an otherwise healthy one", varicocele correction cannot be raccomanded [15]. However some Italian authors reviewed this study and concluded that its statistic methods were poor and its conclusions lacking of significance [16]. *In contrast with Evers and Collins's meta-analysis our study shows a significant improvement of either sperm concentration (82% of patients), and motility (73%). 67% of our patients underwent an improvement of both parameters. Each grade of varicocele was associated with a significant improvement in sperm concentration and most of them also in motility and morphology.*

Age is often studied as a variable related to difference in seminal response to varicocele correction. Although several studied demonstrated a correlation between increase in patients age and decrease of seminal parameters improvement [17 18], this correlation could result from the physiological decline of sperm quality due to age progression, showing no significant differences between seminal improvement and patients' age [19]. *In agreement to this statement our study showed no statistically significance on either improvement differences of seminal paramenters on different-aged patients, and no statistically significance on the linear correlation research of preoperative and postoperative parameters related to patients' ages (p > 0,4).*

Clinical studies focused on the outcome's differences in treatment of subclinical *vs*. clinical varicoceles. Although most of them agreed in treating only clinically evident varicoceles [20 21] some authors demonstrated the effectiveness of treatment of subclinical varicoceles too [5 22 23]. Our study clearly demonstrated that treatment of both subclinical and clinical varicoceles produces a significant improvement on seminal parameters, but no significant difference is evident among the two groups. On the contrary there is a significant difference in sperm concentration improvement among treated patients with absence of venous basal reflow at the CDU investigation (grade 1, 2, 3) and patients with presence of venous basal reflow from renal vein (grade 4, 5).

Conclusion

Results obtained by our study clearly indicate that correction of varicocele can be useful in treating infertile men with both clinical and subclinical varicocele, and no limitations on patients' age should be applied: in fact even patients more than 40 yo can obtain a good improvement in seminal parameters. However the main predictive factor of a better seminal response to varicocele correction is the CDU preoperative evidence of a venous renal basal reflow, according to the pathogenetic hypothesis that testicular damages are mainly caused by venous reflow from renal vein.

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Sexual rehabilitation after nerve sparing radical retropubic prostatectomy: a randomised prospective study on vacuum device *vs.* alprostadil

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Summary

Objective. To evaluate the efficacy of rehabilitation of sexual function in pts. who underwent Nerve Sparing Radical Retropubic Prostatectomy (NS-RRP) for prostate cancer.

Materials and methods. 51 patients underwent NS-RRP. After surgery patients were randomised in two different rehabilitation treatments or included in a control group: group A was treated with alprostadil and sildenafil; group B with vacuum device and sildenafil; group C did not receive rehabilitation. After treatment patients were reassessed by sexual history and IIEF, and data compared.

Results. 12 patients (63%) from group A and 11 (68%) from group B reported spontaneous recovery of sexual activity. Mean IIEF score was 24 for group A and group B, whereas for group C was 9.5.

Conclusions. The group B showed the same rate of recovery of erections of the group A. The use of vacuum represents a valid alternative to alprostadil during rehabilitation and also after rehabilitation has been completed.

Keywords

Radical prostatectomy • Nerve-sparing • Vacuum device • Alprostadil

Introduction

RRP is a treatment options for localised prostate cancer and presents two main complications: incontinence and erectile dysfunction. A nerve-sparing procedure could be performed in localised disease to preserve neurovascular bundles. Postoperatively, despite preservation of the bundles, all patients have a period without erections due to neurogenic shock (neuropraxia).

Recovery of nervous fibres activity takes between 6 and 18 months with a range of 41 to 69% and it's very important to consider all factors that could influence it: age, comorbidity and preoperative erectile function [1 2].

Many authors demonstrated the role of early intracavernous rehabilitation using alprostadil: it helps in preventing fibrotic damages when started shortly after surgery. Furthermore, using 5-PDE inhibitors it's possible to ensure an "endothelial" rehabilitation [2 3].

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Vacuum device is commonly accepted as an alternative to alprostadil in rehabilitation, although there are currently few randomised studies in literature showing its efficacy after RRP [4].
The goal of this randomised prospective study is to verify if vacuum device is so effective and useful as alprostadil for rehabilitation.

Materials and methods

Between December 2003 and December 2007, 51 patients underwent mono or bilateral NS-RRP for localised prostate cancer; all of them signed an informed consent form to participate at the study.
Preoperative assessment consisted in sexual history, IIEF questionnaire (questions 1-5 and 15, score range 0-30) and penile colour Doppler ultrasound using 10 mcg of alprostadil, we considered normal a PSV values higher than 35 cm/sec and a dyastolic value < 5 cm/sec.
One month after surgery, patients were randomly assigned to the two treatment groups (A and B) or in a control group (C):

Group A: intracavernous alprostadil 10 mcg, 3 times a week for 4 weeks, and 50 mg of sildenafil 3 times a week for the following 8 weeks;

Group B: vacuum device daily (15 minutes, 3 times a day) for 4 weeks, followed by 50 mg of sildenafil 3 times a week for a period of 8 weeks.

Group C: no rehabilitation.

The groups were comparable in terms of age, disease, surgical procedure and co-morbidity.
All patients were free to have sexual intercourse. At the end of rehabilitation, after 3 months, the patients were reassessed by sexual history and IIEF questionnaire.
We performed a statistical analysis using IIEF score questions 1-5 and 15 (erectile function). The Kruskal-Wallis test with Bonferroni's correction of post-hoc comparisons, Mann-Whitney test and Wilcoxon test were respectively used in unpaired and paired discrete data analysis. The significance level was set at $p < 0.05$. All data analyses were performed by using SPSS release 10.1.1 for Windows (SPSS Inc., Chicago, USA, 1999).

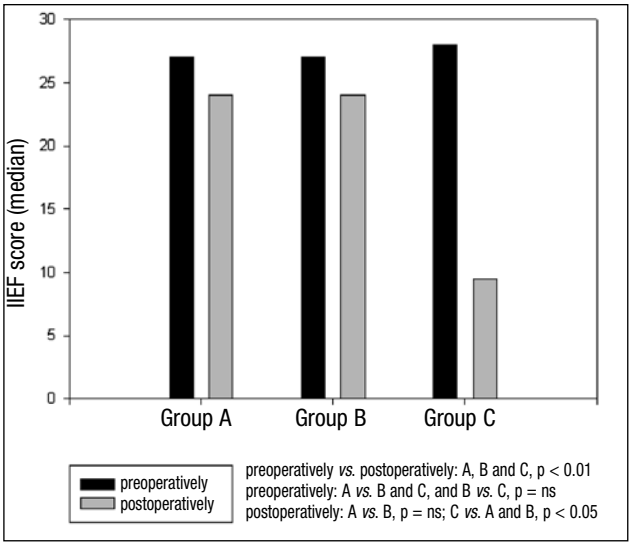
Results

Analysis of the IIEF scores and sexual history revealed that all patients presented with good preoperative sexual function: group A 27, 26-30 (mean age 60.6 ± 5.9 SD), group B 27, 24-30 (mean age 62.9 ± 3.8 SD) and group C 27.5, 25-30 (mean age 62.9 ± 5.9). No patients presented penile vascular disease.
Before starting rehabilitation all patients referred no erections but only tumescences.
After 3 months of rehabilitation, 12 patients in group A showed spontaneous erections (median IIEF score 24, range 23-28) while 7 patients reported satisfying sexual activity using an aid: 5 patients sildenafil while 2 alprostadil (median score 23, range 21-28). In group B, 11 patients showed spontaneous erections (median score 24, range 23-26), whereas 5 patients had satisfying sexual activity using an aid: 2 patients vacuum while 3 sildenafil 100 mg (median score 22, range 21-24) (Table I).
In control group (group C) median IIEF score was 9.5 (range 5-28) and only 3 patients. presented spontaneous erections.
At the end of rehabilitation, there was a significant difference between the two groups who received rehabilitation and the control group ($p < 0.05$) but no

Table I. Recovery of spontaneous erections in the two groups of treatment and patients with sexual activity using an aid.

	GROUP A [PTS.]	GROUP B [PTS.]
Recovery of spontaneous erections	12	11
Sexual activity using sildenafil	5	3
Sexual activity using PGE1	2	0
Sexual activity using vacuum device	0	2

Figure 1. IIEF score before prostatectomy and after postoperative sexual rehabilitation.



significant differences between the two rehabilitation groups (p = NS) (Fig. 1).
About side effects, over 50% of the group A patients referred penile and perineal pain after alprostadil injection, decreasing during treatment.
Patients report no side effects using vacuum, but some of them had some initial difficulties using device.

Discussion

About pathophysiology of ED after RRP: iatrogenic mechanism can essentially be attributed to neurological and vascular damages.
To date, there are no data about preservation of accessory pudendal arteries has some effect on the recovery of sexual function [5 6].
About Neuropraxia there isn't experimental studies on humans; nevertheless, several authors have studied the effect of iatrogenic damage in experimental models [7 8].
As reported in a recent review by Montorsi et al. chronic hypoxia, caused by the reduced arterial blood flow and neuropraxia seem to promote apoptosis of the smooth muscle cells and an increase of connective tissue, leading to fibrosis of the corpora cavernosa [2 9].
This means that patients underwent NS-RRP, need to receive two different rehabilitation: "mechanical rehabilitation" to stretch the cavernous tissues and promote its oxygenation and "endothelial rehabilitation".
Alprostadil and PDE5 inhibitors play a main role in rehabilitation of the corpora cavernosa after NS-RRP, whereas vacuum device has a secondary role, despite it's commonly used in clinical practice. Vacuum device helps passive corpora cavernosa distension making penile structures more elastic and preventing fibrosis. This device is well accepted, can be used several times everyday and represents a valid alternative to alprostadil (group A 63% *vs*. group B 68%; p = n.s.).
Alprostadil has rather frequent side effects such as fibrosis, priapism and painful erections; many patients delayed rehabilitation because they were scared from penile injections. Moreover, the cost of vacuum rehabilitation is comparable to alprostadil, and patiens no need for other money if continues to require an aid.
PDE5 inhibitor play an immediate role in endothelial rehabilitation also if results can be evaluated only over long term [10] but could also used "on demand" among patients who require an help for improving unsatisfying erections.

Conclusions

The data from this randomised prospective study highlights the efficacy of alprostadil for rehabilitating patients with erectile dysfunction after RRP. Vacuum device is a good choice, because efficacy and cost are comparable to alprostadil; furthermore vacuum device encounters greater patient compliance.
Our study confirms the validity of sildenafil in the second phase of rehabilitation, as it guarantees effective continuation of treatment in terms of endothelial rehabilitation once the early and necessary mechanical rehabilitation phase has been completed.

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Evaluation of female sexual function after vaginal surgery with the FSFI (Female Sexual Function Index): our experience

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Summary

Introduction. Urinary Incontinence (UI) and Pelvic Organ Prolapse (POP) have a detrimental effect on Female Sexual Function (FSF). We evaluated the effect vaginal surgery for UI and/or POP on FSF.

Material and methods. The FSFI questionnaire was given to 73 women (mean age of 62 years) undergoing the following operations: 55 Tension-Free Vaginal Sling (TFVS), 12 Kelly plication, 3 hysterectomy + Kelly, 2 Tension-Free Vaginal Sling + Kelly, 1 hysterectomy + Kelly + posterior IVS.

Results. Thirty-six women didn't answer, while 6 were sexually inactive and answered partially; 31 patients answered completely the questionnaire. Pre-operatively, 9 patients had a normal score, while 22 other ones had pathological scores. Mean pre- and post-operative scores were, respectively, 25.26 and 25.22 (normal > 26.55). The FSFI score did not change postoperatively in 26 women, worsened in 3 and improved in 2 who were cured from coital incontinence.

Conclusions. Vaginal surgery for UI and/or POP does not change FSF in the great majority of cases. Worsening or improvement are possible, with cure of coital incontinence being a cause of significant score increase. The high number of patient not answering the questionnaire deserves further studies and it could be – at least partially – explained on the basis of psychological and/or cultural problems regarding the highly emotional issues of sex, incontinence and prolapse.

Keywords
Vaginal surgery • Female Sexual Function • Incontinence • Pelvic organ prolapse

Introduction

It is well known and generally accepted that both urinary incontinence (UI) and pelvic organ prolapse (POP) have a detrimental effect on female sexual function (FSF) [1-11]. Their successful surgical correction should improve the patients' sexual life, but, as a matter of fact, such improvement has been never demonstrated [12]. Moreover, vaginal surgery may be a cause of sexual dysfunction (SD): anatomical and functional modifications, psychological consequences and surgical complications may occur [12-16], and all of them may have a negative effect on sexual life.

At present, the basic question we are faced with is which is the effect

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of surgery of UI and/or POP on female sexuality. The purpose of the present study was to evaluate the FSF in those women undergoing surgical treatment fro UI and/or POP operated at our Centre.

Material and methods

The FSFI (Female Sexual Function Index) questionnaire is based on 19 questions dealing with 6 components of FSF: desire, arousal, lubrication, orgasm, satisfaction, and pain [17 18]. A score of 26.55 has been defined as the optimal cut score between normal and pathological values [19]: sexual life was considered normal in the patients with score higher than 26.55 and pathological in those with lower values. The changes possibly induced on FSF by vaginal surgery were evaluated by means of the comparison between pre- and post-operative FSFI score values.

At the moment of the post-operative FSFI administration, all women were subjected to a clinical interview and to a physical urogynecological examination, both aimed at the evaluation of the surgical success rate.

Results

The FSFI questionnaire was administered to 73 women, whose mean age was 62 years before and after vaginal surgery aimed at the correction of UI and/or POP.

Specifically, the patients underwent the following procedures: 55 TVT for the correction of stress urinary incontinence; 12 anterior colporrhaphy with Kelly plication for anterior vaginal wall prolapse; 2 TVT and Kelly plication; 3 vaginal hysterectomy (for grade III or IV uterine prolapse) and Kelly plication; 1 hysterectomy, Kelly plication, and posterior IVS for the correction of vaginal vault prolapse.

Thirty-six patients did not complete the preoperative questionnaire. Their mean age was 65 years and they underwent TVT in 23 cases, Kelly plication in 9 cases, vaginal hysterectomy and Kelly plication in 3 cases, TVT and Kelly plication in a single case, and hysterectomy, Kelly plication, and posterior IVS in a single case, respectively.

Six patients (4 undergoing TVT and 2 Kelly plication) reported to be sexually inactive and answered the questionnaire limitedly to the specific domains of desire, arousal and lubrication.

Thirty-one patients filled completely the questionnaire. Their mean age was 54 years. Among those, 29 had TVT, 1 Kelly plication, 1 TVT and Kelly plication. Their mean follow-up was 15 months (range 3 to 24 months).

The mean preoperative FSFI score of these 31 women was 25.26 (range:16.3-35.1). In only 9 of them (8 subjected to TVT, and 1 to Kelly plication) the preoperative FSFI score was normal. Their mean age was 52 years and the mean score 30.1. In 8 of these 9 women the FSFI score remained unchanged after the treatment, while in a single one undergoing TVT it worsened but remained higher than 26.55.

Twenty-two (21 subjected to TVT and one to TVT and Kelly plication) had a pathological preoperative FSFI score: their mean age was 55 years and their mean score was 23.28. In 18 of them the score remained unchanged after surgery, while, among the remaining 4 all undergoing TVT, the score worsened in 2 cases and improved in 2 further ones (in one of them shifting to a value higher than 26.55).

Anterior vaginal prolapse was totally cured in 2 cases by the Kelly procedure.

No major complication was reported; 2 transitory urine retentions were quickly and definitively solved with clean intermittent catheterisation. All the 30 women operated for SUI reported good results: 27 were totally satisfied, 2 complained of occasional and slight mixed urinary incontinence and a single one had a significant improvement of her incontinence, despite showing a residual SUI which needed the use of a single pad/day, compared to the 3 ones she used before surgery.

The mean post-operative score was 25.22 (range: 14.6-35.1). FSFI showed no change in 26 patients (83.9%), in which pre- and post-operative scores were the same (25.8 ± 4.9). 24 of them had been subjected to TFVS, 1 to Kelly plication, 1 to TVFS and Kelly plication.

Tree patients (9.7%) subjected to TVT, aged 53,42 and 48, had a postoperative decrease of their FSFI, with scores changing from 17.6, 24.6 and 30.3 to, respectively, 14.6, 23.6, 28.7. The latter patient frankly reported the fear that intercourse could damage the good results of TVT sling.

Two patients (6.4%), aged 55 and 61, reported an improvement of their FSFI score (from 16.3 and 23.6 to 17.2 and 27.3): both suffered from a coital incontinence which was cured by TVT, the latter patients being the only one in our experience where the FSFI score shifted from a pathological to a normal value.

Discussion

Our study demonstrated that vaginal surgery for UI and/or POP did not change FSF in the great majority

of cases. However, some patients reported worsening of the preoperative sexual function, while those who were cured from coital incontinence experienced improvement in their scores.

In the field of surgery for UI and/or POP, the impact of treatment outcome on patients quality of life and sexual function should be a major issue of interest. Among the different questionnaires aimed at the evaluation of FSF, we decided to use the FSFI because, with its 19 questions on 6 well defined domains, it could be yet considered among the most comprehensive questionnaires for the evaluation of female sexuality. Nevertheless, we are well aware that a complete questionnaire for the evaluation of FSF is yet to be found: female sexuality, involving anatomical, biological, psychological, interpersonal and social components, is very difficult to evaluate, certainly more than male sexual function.

In our opinion, all the available questionnaires (including the FSFI) aimed at the evaluation of FSF are nowadays fashioned on masculine models, and are not completely corresponding to real life.

In our experience, 35 out 72 patients (48%) did not answer the questionnaire, which deserves certainly some considerations. In the other published reports, the number of patients who refused to answer questions about sex after surgery ranges ranged from 15% to 25% [13 16 20]. All those studies used less extensive questionnaires, including 3 to 9 questions directly addressing sexuality. In our experience, moreover, the patients who didn't answer the questionnaire were significantly older than those who responded and had a higher number of POP. It is well known that FSF worsens with age [21], and the relationship between age, high-grade POP and absence of intercourse has been already documented [22 23]. Notwithstanding, 6 sexually inactive patients with a mean age of 60 decided to answer the questionnaire, showing then interest and lack of inhibition toward sexuality. It could be then reasonably hypothesized that, behind the refusal of 35 women to fill the questionnaire, there was not only the absence of sexual life, but also some kind of psychological and/or cultural issues related to the highly emotional issues of sexuality, incontinence and prolapse.

Our experience and the current literature confirm [1-11] that UI and/or POP have a detrimental effect on FSF in the majority of cases: the mean pre-operative score of our 31 women was pathological (25.26), and the score was pre-operatively impaired in 22 of them (72%). There was no significant mean age difference between women with pathological and normal score (55 *vs*. 52 years).

Owing to the very small number of patients with POP who answered the questionnaire (n = 2) we were unable to draw clear conclusions about the impact of the surgical correction of POP on FSF.

Vaginal surgery for incontinence causes no change in FSF in the great majority of cases. The post-operative score was very slightly, insignificantly lower than the pre-operative one (25.22 *vs*. 25.26). After surgery, the FSFI score remained completely unchanged in 26 women (83.9%), changed slightly in 4 (3 worsenings, 1 improvement) without shifting from normal to pathological or the opposite, and only in one case shifted from one type of score (pathological) to the other (normal). These data are in agreement with most of the papers so far published [4 13 16 24 25], who reported that female SF is not changed by vaginal surgery, either for UI or POP, in the majority (from 62% to 100%) of cases [26-28].

Impairment of FSF after surgery for UI and/or POP has been reported in many papers and with percentages rarely exceeding 20% of the patients [4 13 16 20 25 27-29]. In our experience, only 3 women (9.7%), subjected to TVT, had a post-operative decrease of the FSFI score. In the only one of them who had a normal preoperative FSF (the patient reporting fear that intercourse could damage the results of surgery), the score remained normal after the operation.

The causes of FSF deterioration after urogynecological surgery may be divided [15] into organic, emotional and psychological. Organic causes can be further divided into anatomical, physiological, hormonal, neural and vascular. A vaginal narrowing, mostly as a consequence of perineorrhaphy or posterior colporrhaphy [15 26 30], may cause dyspareunia; on the contrary, there is no reported association between vaginal length and FSF [15 30], and also the available data regarding FSF after hysterectomy are controversial [15 31 32]. Blood vessels and neural terminations are located mainly on the anterolateral vaginal walls and along the urethral walls, so that they can be damaged during urogynecological surgery: decreased vaginal sensitivity and lubrication may subsequently occurr [6 33-35]. Surgical failures and/or complications such as tape erosion [13 15] may impair FSF but they were not reported in our experience and the FSF worsening observed in our patients cannot be attributed to them.

The highest percentage of postoperative FSF improvement so far reported slightly exceeds
30%; in our experience, only 2 patients with pathological score (6.4%) had a postoperative improvement, and only in one of them a shifting to normal values was observed. Both patients had a coital

incontinence which was totally cured by TVT. UI during sexual intercourse is a well-known problem [6 8], which was [4 25 36] reported to be present up to almost half of the women affected by UI. Every type of incontinence may be associated to sexual activity; the loss of urine may happen not only during penetration but in some cases during preliminaries and/or orgasm [36]; considering all patients with a reported post-operative FSF improvement, the percentage of those who had a solution of a coital incontinence ranges from 50% [4] to more than 90% [25].

The results of the treatment can be considered satisfactory, thus confirming that vaginal surgery is a steady acquisition of all surgeons who practice it regularly. Postoperative FSF does not seem to be affected, at least in our experience, by the surgical outcome. Obviously, the risk that a surgical failure could significantly worsen sexuality can be never ruled out.

Conclusions

Our experience seems to confirm the assumption that UI and/or POP have a detrimental effect on FSF. Few women with POP answered the questionnaire, but, on the other hand, 22 out of 31 women had an impaired FSF before treatment. Moreover, our experience seems to indicate and confirm that vaginal surgery guarantees good success rates in the correction of SUI and POP.

There is a need of more adequate and standardized instruments for the definition of success, failure and improvement after surgery for UI and/or POP, as well as for the evaluation of the impact of this kind of surgery on FSF and quality of life.

Vaginal surgery does not seem to change FSF in the great majority of cases but worsening or improvements may occur, but they rarely reach percentages exceeding 20%. Despite the general cultural progress and the increasing awareness about general and sexual health, too many women are yet reluctant to discuss about sex, incontinence and prolapse. The existence of a psychological and/or cultural problem can be reasonably hypothesized for many of them: a cultural battle against prejudice and unmotivated shame is probably yet to be fought in our everyday practice.

All professionals involved in the interdisciplinary field of urogynecology should be committed to the early identification of patients with these problems: early diagnosis and adequate treatment could spare to many women the progressive deterioration of their FSF caused by potentially curable conditions like UI and/or POP. Surgery is indeed nowadays highly successful, safe and in some cases mini-invasive.

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Dear Editors,
I am little surprise about the article in the first issue of Journal of Andrological Sciences that I considered our Journal. At page 181 there is a nice paper about a SIA compaign which I took part. The Authors reported that “100 andrologists partecipated in this campaign” and “the number of phone calls received on the free help line totalled 14,649 in total with 4,951 received just within the first 24 hours. Andrologists answered 11,109 phone calls ...”.
I am surprise, also beeing a member of SIA, about the fact that there is not a list of all the Andrologists that helped to build this report with such a great work. This issue was also discussed during the general assembly in Rome with Professor Gentile that was informed about a certain grade of dissatisfaction of the members.
I strongly suggest, for the next issue, to publish a list of all members who did this hard work.

Best regards
Federico Dehò

Dear Dr. Dehò,
I appreciated your letter to the journal and I completely agree with you concerning the opportunity to report all the andrologists participating in the campaign “a return to love without worry”.
At the same time, I do remember that this critical issue has been highlighted during the general assembly in Roma. For those reasons, I ask the SIA executive committee to published the complete list of participants of the campaign in the SIA corner .

Yours sincerely
Vincenzo Ficarra

List of Practitioners who adhered in 2008 to the campaign “Amare senza Pensieri”

http://www.amaresenzapensieri.it/campagna.aspx

Antonini Gabriele
Barrese Francesco
Battiato Carmelo
Belgrano Emanuele
Belgrano Giovanni
Benazzi Emanuele
Benedetto Giuseppe
Bertozzi M.Antonella
Bettocchi Carlo
Biagiotti Giulio
Bianchessi Ida
Bianchi Bruno
Bonaffini Cristina
Bonanni Guglielmo
Branchina Antonino
Bruzziches Roberto
Bulzomì Rocco
Calabrese Massimo
Caldarera Emanuele
Campo Salvatore
Capone Massimo
Caraceni Enrico
Cardella Antonino
Casarico Antonio
Ceruti Carlo
Colombo Fulvio
Contemori Giampaolo
Cozza Pietro Paolo
Curreli Andrea
D’Amico Andrea
D’Anzeo Gianluca
Dadone Claudio
De Grande Gaetano
De Rose Aldo
Dehò Federico
Del Noce Giorgio
Di Palma Paolo
Diambrini Maurizio
Ferrini Fausto
Franco Giorgio
Fusco Ferdinando
Gattuccio Ignazio
Gentile Vincenzo
Giambersio Antonio
Giammusso Bruno
Giuffrida Concetto
Granata Antonio Maria
Guerani Attilio
Iatrino Giuseppe
Ilacqua Nicola
Iurato Carlo
La Vignera Sandro
Lanzafame Francesco
Lauretti Stefano
Maio Giuseppe
Malvestiti Mario
Mancini Mario
Mantovani Franco
Maretti Carlo
Marzotto Caotorta
Mastroeni Francesco
Mereu Eugenio
Michetti Paolo Maria
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Mondaini Nicola
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Paulis Gianni
Pavone Carlo
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Leydig cell tumor or adrenal rest tumor of the testes? A case of uncertain diagnosis

R. Boscolo-Berto[*], E. Bonandini[**], M. Gardiman[**], V. De Marco[*], M. Iafrate[*], G. Novara[*]

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Summary

Congenital defect of 21-alpha hydroxylase is a common enzymatic defect subtended to a cortisol synthesis deficiency, and is a usual feature of the clinical picture named adrenogenital syndrome.
Testicular tumors in the adrenogenital syndrome are an uncommon benign disease including multiple, bilateral and usually synchronous nodules rising inside the testis when the steroidal function of the adrenal gland cortex was deficient.
Testicular tumors resistant to a medical approach and associated with the adrenogenital syndrome have traditionally been managed with a tumor enucleation or partial orchiectomy in order to exclude a malignant disease. The testicular lesions are often mistaken for Leydig cell tumor, nevertheless the behavior of these latter neoplasms is significantly different with up to 10% of them being malignant.
We present a case of bilateral nodular hyperplasia of the testis without adrenal hyperplasia in a patient affected by 21-alpha hydroxylase deficiency. This mass mimicked a testicular tumor and made differential diagnosis with a Leydig cell tumor extremely difficult on histological basis. Some immunophenotypic features, including synaptophysin staining, were useful to distinguish testicular tumor of the adrenogenital syndrome from Leydig cell tumor, avoiding a misdiagnosis potentially impacting on patient prognosis.

Keywords
Adrenogenital syndrome • Leydig cell tumor • Testis tumor

Introduction

Testicular tumors in patients with adrenogenital syndrome are rare but well documented in medical literature [1-5]. Adrenal type nodules arise bilaterally inside the testicular tissue when the function of the adrenal gland cortex was deficient [6]. Congenital defect of 21-alpha hydroxylase is characterized by a deficiency of cortisol synthesis, high levels of serum testosterone due to peripheral conversion of increased adrenal androstenedione and reduced aldosterone production, leading in most of the cases to a salt-losing syndrome [7]. The lack of cortisol-negative feedback to the pituitary gland causes the rising of ACTH synthesis with a hyperplasia of ACTH sensitive tissue both in the adrenal gland and in other sites, such as testis, in which adrenal foci can be detected [8]. Testicular masses seldom develop, with patients experiencing a lack of response to pharmacological therapy being at

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higher risk. These masses are usually bilateral and synchronous. Some hypotheses have been done to explain the histological origin of such nodules. They may derive from adrenal cells migrating to the scrotum during the physiologic descent of the testicle or from a totipotential stem cell in the testicular interstitium, giving rise to the Leydig cells that are able to differentiate into adrenocortical cells [6 8]. The lesion is often mistaken for Leydig cell tumor (LCT) [9]. However, the behavior of these neoplasms is significantly different with up to 10% of LCTs being malignant [10]. Although tumors of adrenogenital syndrome may regress in consequence of systemic administration of exogenous steroids, testicular tumors resistant to a medical approach and associated with the adrenogenital syndrome have traditionally been managed as true neoplasms with a surgical intervention consisting of tumor enucleation or partial orchiectomy in order to preserve fertility, control local symptoms, and scrotum anatomy [11-13].

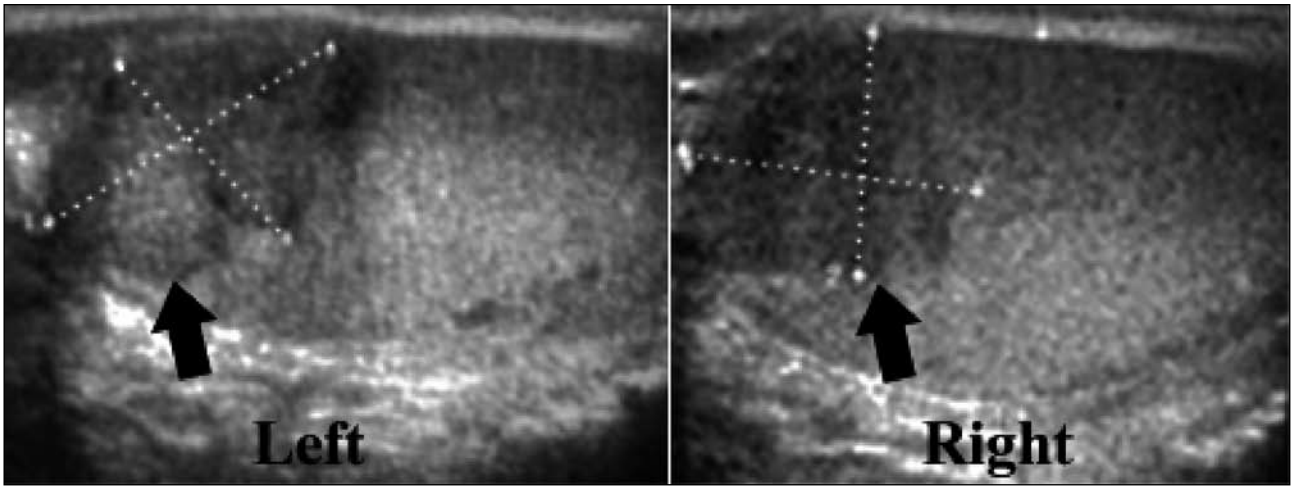
We report a case of synchronous testicle tumor in which the differential diagnoses between Leydig cell tumor and tumor of adrenogenital syndrome was extremely difficult.

Case report

A 30-year-old male was admitted to our Urology Clinic with a suspect for bilateral synchronous testicular neoplasm in a patient with a 21-hydroxylase deficiency implying an adrenogenital syndrome without salting-lost features diagnosed in childhood. He reported the synchronous and progressive appearance of bilateral testicular masses, during substitutive medical therapy with dexamethasone at doses of 0.375 mg/die, fludrocortisones acetate at 0.1 mg/die, cortone acetate at 12.5 mg/die. On admission, he had normal laboratory values and a poorly compensated hormone profile: ACTH, 450 pg/mL (normal range 10-50 pg/mL); 17-OH progesterone 258 nmol/L (1.5-6.4 nmol/L); androstenedione 19.9 nmol/L (2.1-10.8 nmol/L); testosterone was normal and the patient was oligoasthenozoospermic. At physical examination, a systemic melanodermia was noted, while the external genitalia appeared of normal size and shape. He referred normal growth and psychosexual development. Hard nodules 1-cm and 1.5-cm large were present at the level of the right and left testis, respectively. The patients did not have any testicular discomfort or pain. These nodules were confirmed by a scrotal ultrasound as hypoechoic masses located on the upper pole of both the testis. Nuclear magnetic resonance of the upper abdomen showed normal-sized adrenal glands. Despite the administration of a stronger suppressive medical treatment with exogenous steroids, at the next follow-up a scrotal ultrasound did not show any reduction in the size of the testicular masses and the hormone profile still remained poorly responsive to the compensation (Fig. 1). Considering the possibly malignant origin of testicular masses, a surgical intervention was scheduled.

At surgery, a hard, lobular nodule of about 1.0 cm was identified into the upper testicular pole, under the tunica albuginea of testis that appeared otherwise normal. On the left side, a similar 1.5 cm node was identified in the upper testicular pole. Both lumps were enucleated and, macroscopically, they appeared light brown. The pathological diag-

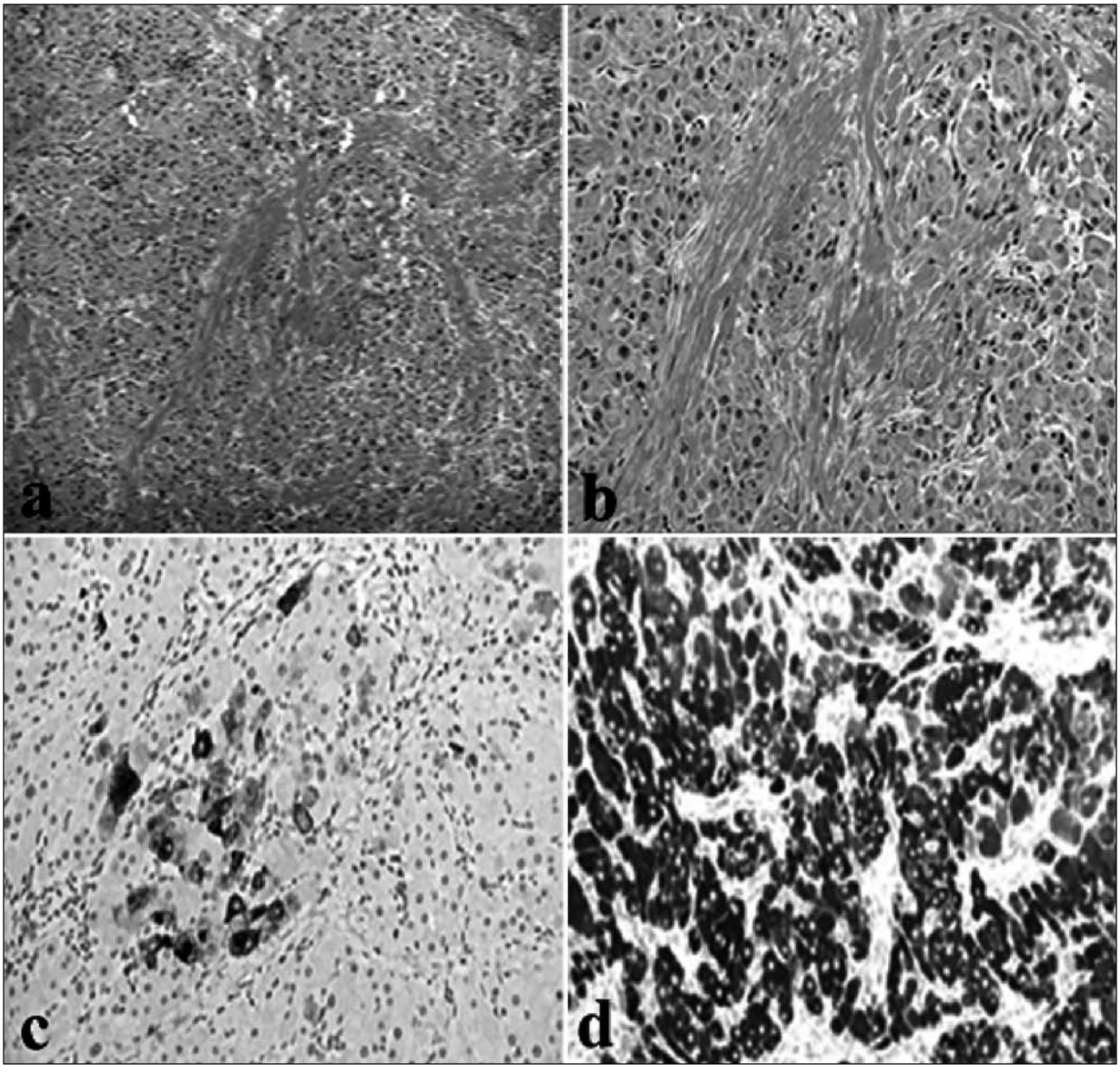
Figure 1. Scrotal ultrasound showing hypoechoic heterogeneous lumps located on the upper pole of both the testis. The left one was about 1.5 cm large, and the right one was 1.0 cm large. There was no significant increase in testis volume.



nosis on frozen section was of benign tumors, with morphological features compatible with a tumor of adrenogenital syndrome or LCT. To obtain the final histological diagnosis, immunohistochemical analyses using antibodies against inhibin A (R1 clone; Dako) and synaptophysin (SY38; Dako) were done, other than the traditional hematossilin-eosin staining. Microscopically, tumors consisted of polygonal cells with large eosinophilic cytoplasm and mild nuclear pleomorphysm. Some lymphoid aggregates and septa of fibrosis were also seen. Mitotic activity, lipochrome pigment and adipose metaplasia were absent. Also Reinke crystalloids were not seen (Fig. 2a, 2b).

Immunohistochemical analyses showed an intense and widespread staining for inhibin alpha, with a strong and patchy reactivity for synaptophysin (Fig. 2c, 2d). The strong staining for synaptophysin leaded to the final report of tumor of the adrenogenital syndrome, as described by Ashley and colleagues [14].

Figure 2. Histopathological and immunohistochemical evaluation. 2a. Hematossilin-Eosin staining (x100), showing absence of adipose metaplasia and lipochrome pigments, with presence of extensive fibrosis and lymphoid aggregates. 2b. Hematossilin-Eosin staining (x200), showing absence of severe nuclear pleomorphysm and lack of mitotic activity and Reinke crystalloids; 2c. Immunohistochemical staining (x200), showing patchy reactivity for synaptophysin; 2d. Immunohistochemical staining (x200), showing wide spread strong reactivity for inhibin A.



Discussion

The adrenogenital syndrome includes a group of autosomal recessive defects in the field of adrenal steroidogenesis, the most common of which is represented by the 21-hydroxylase deficiency [15]. The clinical syndrome in male individuals includes early virilization of the external genitalia and symptoms associated with steroid deficiency, usually including lethargy, emesis, diarrhea, hypotension with dehydration and failure to thrive that ultimately stunts height in older children with an earlier rapid somatic growth. Despite these peculiar presentations, the adrenogenital syndrome may not be recognized in its milder forms, and sometimes is uncovered investigating the onset of bilateral testicular masses as clinical expression of up to 18% of cases [6]. In these patients, without a previous diagnosis of adrenogenital syndrome or a concomitant clinical suspicion, there is a possibility of a pathological misdiagnosis with Leydig cell tumor. Leydig cell tumors represent about 3% of all testicular tumors [16]. In childhood the masses are usually unilateral and they present between the 4 and 5 year of age, and only 3% of Leydig cell tumors develop bilaterally differently form the 83% incidence of bilateral tumors associated with the adrenogenital syndrome [6 16]. Because of a considerable pathological similarity, a complete biochemical and histopathological evaluation is required to make the correct diagnosis, which may represent a real challenge [9 17 18].

Testicular lesions in the adrenogenital syndrome are hormone dependent and thus are not considered true self-standing tumors. In a description of the benign behavior these masses were labeled tumors of the adrenogenital syndrome [6]. Adrenal rests, interstitial cells, and pluripotential cells of the testicular stroma stimulated by elevated levels of ACTH have been considered as possible origins of the tumors found in adrenogenital syndrome [2 8 19]. Adrenal rest tissue is described along the normal path of testicular descent in 50% of newborns [20]. Adrenal remnants are usually found in some extratesticular locations, such as within the connective tissue of the spermatic cord, contiguous to the epididymis or rete testis, adjacent to the hilum of the testis [6], while they are less common within the testis, even though clearly described in 7.5% of cases [21]. In children with the adrenogenital syndrome some Authors found testicular nodules in all subjects older than 14 months [22]. Usually adrenal rest tissue involves during early infancy, while in the adrenogenital syndrome the elevated ACTH level stimulates such adrenal remnants to respond with hyperplasia, leading to an increased testicular size or the development of testicular masses [22]. Biochemical profiles should include measurement of 17-hydroxyprogesterone, 11-desoxycortisol, dehydroepiandrosterone, androstenedione and testosterone levels [18]. Testosterone is the major androgen that is increased to adult levels in Leydig cell tumors, while testosterone is not elevated in patients with tumors of the adrenogenital syndrome.

When the biochemical profile is ambiguous and steroid levels do not clearly define the tumor of origin, dexamethasone suppression and adrenocorticotropic hormone stimulation tests may assist in the correct diagnosis [16 20]. The suppression of elevated adrenocorticotropic hormone by replacement steroid therapy usually causes rapid regression in tumor size in up to 75% of cases [6], even though sometimes the dose of steroid required to cause tumor regression was considerably greater than that sufficient to correct the underlying biochemical defect [11]. Nevertheless, some testicular tumors still fail to regress despite therapy, raising the clinical doubt of an unresponsive tumor of adrenogenital syndrome or the occurrence of a LCT.

Traditionally surgical orchiectomy has been the standard treatment for tumors of not clarified origin or of steroid resistant tumors in patients with the adrenogenital syndrome [12 13]. Our histological findings were ambiguous, since they showed the absence of adipose metaplasia, severe nuclear pleomorphysm and lipochrome pigments as in the majority of LCTs, but the presence of extensive fibrosis, lymphoid aggregates and a concomitant lack of mitotic activity and Reinke crystalloids as in tumors of adrenogenital syndrome (Fig. 2a, 2b).

The testicular tumor of the adrenogenital syndrome may show evidence of fatty metaplasia, causing potential confusion with LCT having adipose differentiation [23 24]. Some Authors clearly reported some features of adrenal rest tissue, including multiple extratesticular nodules and the presence of cells with vesicular nuclei but without Reinke crystals and with a cord-like arrangement that mimics the adrenal cortex [11]. Crystalloids of Reinke are found in only 30 to 46% of Leydig cell tumors, while they are lacking in tumor of adrenogenital syndrome [14 25]. Overall, our patient didn't match all the morphological criteria allowing the pathologist to report a diagnosis of LCT instead of tumor of adrenogenital syndrome.

Recently some Authors have demonstrated the feasibility of testis sparing surgery as an alternative to traditional orchiectomy in cases of benign tumors of the testis [12 13]. The main concern with salvage procedures of any organ is the possibility of local tumor recurrence or distant spread. It's reported that up to 10%

of Leydig cell tumors in adults are malignant, with a clinical expression in older patients. Surgical enucleation with testicular preservation is also described as an effective management of testicular neoplasms associated with the adrenogenital syndrome without evidence of recurrence after 48 months of follow-up [11 12]. To date, there are no reported cases of metastatic disease with interstitial cell tumors or tumors of the adrenogenital syndrome in children [4 6]. On this basis the patient is considered healed, and entered a program of periodical follow-up.

Conclusion

This patient presented with a bilateral synchronous testicular mass mimicked a testicular tumor of uncertain origin as it did not show any reduction in the size of the testicular masses and the hormone profile still remained poorly responsive to the steroidal suppressive therapy. Considering the possibly malignant origin of testicular masses, a surgical intervention was scheduled. On microscopic histological analysis, the differential diagnosis with a Leydig cell tumor was extremely difficult and the use of immunophenotypic features, including synaptophysin staining, allowed to diagnose a testicular tumor of the adrenogenital syndrome.

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Milla PJ. Electrogastrography in childhood: an Overview. In: Chen JDZ, McCallum RW, eds. Electrogastrography Principles and Applications. New York: Raven Press Ltd 1994, p. 379-96.

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