Doppler ultrasound of the testis in azoospermic subjects as a parameter of testicular function

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Azoospermia frequently represents the end-point of different pathological conditions that cause important quantitative and qualitative alterations of both spermatogenesis and testicular structure, including intratesticular blood vessels. In this study we performed colour Doppler ultrasound of the testis in 12 azoospermic subjects affected by primary testicular pathology (four bilateral post-orchitis, four post-radiotherapy for cancer, four post-traumatic) aged 28.2 ± 3.3 (mean ± SD) years, in six subjects affected by obstructive azoospermia aged 29.7 ± 2.4 years and in 20 age-matched fertile subjects (aged 28.6 ± 2.5 years). The analysis of intratesticular vessels per organ was quantified using a semiquantitative score: category 0, no vessels visible; category 1, between one and three intratesticular vessels visible; and category 2, more than three vessels visible. In obstructive azoospermic patients and in fertile subjects there were always more than three intratesticular vessels. No intratesticular vessels were detected in eight testes (33.3%) and fewer than three vessels in 16 testes (66.6%) in subjects affected by primary testicular pathology. In azoospermic subjects the testicular structure of the testis was evaluated by diagnostic fine needle aspiration cytology (FNAC) performed in the middle portion of the testis. In non-obstructive azoospermic patients this procedure showed the presence of only Sertoli cells in all cases. When detectable vessels were present, a new aspiration was performed in these areas. In 12 out of 16 cases, spermatogenetic cells including mature spermatozoas were found when the FNAC was performed in testicular regions showing the presence of blood vessels. These results indicate that colour Doppler sonography of the testis may be useful in the differential diagnosis of azoospermia and suggest the evaluation of the intratesticular blood vessel distribution before performing any method to retrieve intratesticular spermatozoas for intracytoplasmic sperm injection.

Key words: azoospermia/colour Doppler/spermatogenesis/testis

Introduction
A large number of patients evaluated for infertility (~20–30%) are azoospermic (Foresta et al., 1995). In these cases, when mature spermatozoas or spermatids are present in the testis, intracytoplasmic sperm injection (ICSI) makes fertilization and pregnancy possible through the extraction of these cells from testicular tissue (Jow et al., 1993; Craft and Shrivastav, 1994; Tourney et al., 1996). This procedure can be easily carried out in obstructive azoospermia but when a primary testicular pathology is present the recovery of intratesticular spermatozoas is very difficult or completely impossible.

The testicular structure in azoospermic subjects affected by primary testicular pathology is frequently related to Sertoli cell-only syndrome or to severe hypospermatogenesis showing rare and isolated spermatogenic foci (Foresta et al., 1995). At present there are no parameters (clinical, seminal, hormonal or sonographical) that enable us to identify the presence of spermatogenic tissue within the testis and consequently multiple biopsies are performed until spermatozoas are found (Shlegel and Su, 1997).

In primary testicular pathologies, testicular structures are completely modified and so intratesticular blood flow may be changed. Colour Doppler ultrasound imaging evaluates the intratesticular blood flow (Middleton et al., 1989; Bader et al., 1997) and it has been proposed as a promising method for the assessment of patients with intrascrotal pathological conditions, e.g. testicular torsion or epididymitis, which show a decreased and an increased blood flow respectively (Krieger et al., 1990; Ingram and Hollman, 1994). To evaluate whether testicular blood flow is impaired in the presence of a primary testicular pathology and whether its distribution makes it possible to identify isolated spermatogenetic foci, we performed colour Doppler ultrasound and power colour Doppler ultrasound imaging in testes from a group of azoospermic subjects affected by primary testicular pathology or seminal tract obstruction and in 20 fertile normozoospermic controls.

Materials and methods
The study was approved by the Hospital Ethical Committee of Padova and informed consent was obtained from each patient. We studied 18 patients who were referred to our infertility clinic for azoospermia. A total of 12 subjects (aged 28.2 ± 3.3 years) were affected by primary testicular pathology with a cytological picture of Sertoli cell-only syndrome and high plasma concentrations of follicle stimulating hormone (FSH) (bilateral post-mumps orchitis, n = 4; post-radiotherapy for testicular cancer, n = 4; post-traumatic, n = 4); six subjects (aged 29.7 ± 2.4 years) were affected by obstructive azoospermia with normal testicular cytological picture showing the presence of normal spermatogenesis and normal FSH plasma concentrations. In addition, we studied 20 age-matched fertile men (aged 28.6 ± 2.5 years) as controls.

All patients underwent colour Doppler sonography and power
Doppler ultrasound of the testis in azoospermic subjects

Figure 1. Testicular mapping: 12 possible assignments to discriminate different areas in the testis.

Doppler sonography performed by a 7.5 MHz phased-array transducer (Logiq 500; GE Medical Systems, Milwaukee, WI, USA). Testicular volume was evaluated by using the approximation for a prolate ellipsoid (volume = length x width x depth x 0.523). We also identified capsular, supratesticular and intratesticular arteries. In all patients, scans were obtained in multiple orientations to ensure the imaging of blood vessels, if present, at the angle that maximized the Doppler frequency shift and thus maximized depiction. The analysis of intratesticular blood vessels per organ was quantified using a semiquantitative score: category 0, no visible vessels; category 1, between one and three intratesticular vessels visible; category 2, more than three vessels could be identified. We also determined whether visible intratesticular vessels were located in the medial or lateral, anterior or posterior side of cranial, middle, or caudal third of the testis (12 assignments possible, Figure 1). Plasma concentrations of FSH and luteinizing hormone (LH) were measured by radioimmunoassay using [125 I]-labelled FSH and LH and a double monoclonal antibody (Ares-Serono, Milan, Italy). Testosterone plasma concentrations were determined using a double antibody radioimmunoassay.

In azoospermic subjects the analysis of testicular structure was performed with conventional fine needle aspiration of the testis by a puncture into the middle portion of the testis, on the side opposite to the epididymis (Foresta et al., 1992). Testicular cytology was evaluated as previously described (Foresta et al., 1992). In azoospermic patients affected by primary testicular pathology who did not show germinal cells in the first fine needle aspiration of the testis, a new aspiration was performed after Doppler ultrasound in specific areas showing the presence of blood flow. In azoospermic patients with no germ cells after the first fine needle aspiration of the testis and no intratesticular detectable blood vessels, a re-aspiration of the testis was not performed.

Statistical analysis
Results are given in the text as mean ± SD. Statistical comparisons between groups were carried out by analysis of variance. P < 0.05 was considered to be statistically significant.

Results
In subjects affected by primary testicular pathology, the cytologic testicular picture as evaluated with fine needle aspiration of the testis showed the presence of Sertoli cells only. In these subjects testicular volume was significantly lower than that of fertile subjects (12.3 ± 2.9 versus 15.6 ± 3.1 ml, P < 0.05),
Colour Doppler and power Doppler sonography did not show any difference of supratesticular and capsular vessels in obstructive azoospermia, in non-obstructive azoospermia and in fertile subjects. The comparison of the number of detectable vessels in each testis with colour Doppler versus power Doppler sonography revealed that it was possible to detect the same number of vessels in 100% of testes of patients affected by primary testicular pathology, while in testes of obstructive patients and fertile controls, power Doppler sonography allowed the identification of a greater number of blood vessels than Doppler sonography. However, in these cases, intratesticular blood vessels were always more than three (category 2, Figure 2a). Fewer than three vessels (category 1) were observed in 16 testes (66.6%, Figure 2b) including seven bilateral and two monolateral forms in subjects affected by primary testicular pathology. No intratesticular vessels (category 0) were detected in eight testes (33.3%) including threee bilateral and two monolateral forms (Figure 2c). Figure 3 shows the quantification of intratesticular blood vessels according to testicular volume. The reduction of vessel number detected in non-obstructive azoospermia was not related to testicular volume. In these patients fine needle aspiration of the testis performed in the testicular region corresponding to the detectable vessels, when present, showed the presence of spermatogenetic cells including mature spermatozoa (Figure 4a and b) in 12 out of 16 testes. Only two patients showed no spermatogenetic cells (Figure 4c).

Discussion
Blood flow in the testis is supplied by several arteries derived primarily from branches of the internal spermatic artery with collateral branches of the cremasteric artery (Shlegel and Su, 1997). The testicular blood supply penetrates the tunica albuginea and then travels between the septa separating the seminiferous tubules (Middleton et al., 1989; Martinoli et al., 1992). Colour Doppler sonography and power Doppler sonography represent reliable methods of investigating testicular arteries and have been used in the diagnosis of testicular torsion, epididymo-orchitis and intrascrotal pathologies, e.g. tumours and abscesses. Recent assisted reproductive techniques as well as ICSI may be performed with a single spermatozoon and in both obstructive and in non-obstructive azoospermia, mature spermatozoa can be obtained by testicular biopsy or aspiration. In non-obstructive azoospermia, the retrieval of spermatozoa is very difficult since few areas of spermatogenesis may be present and, at present, their localization is impossible. Recently, Silber et al. (1997) have suggested that a diagnostic testicular biopsy should be carried out in all azoospermic patients undergoing ICSI in order to evaluate the possibility of success in retrieving mature spermatozoa when performing assisted reproductive techniques. To overcome this difficulty, other authors have suggested multiple biopsies or testicular
aspirations and observed the presence of spermatozoa in ~34% of men with non-obstructive azoospermia (Schlegel and Su, 1997). These methods are not feasible in all centres and involve the removal of excessive tissue from testes, raising a number of clinical and ethical concerns about their routine use. The testicular structure in non-obstructive azoospermia is severely altered and the results of this study demonstrate that, in all patients examined, testicular blood flow is also strongly modified showing decreased or absent intratesticular arterial flow. On the contrary, in obstructive azoospermia the testes exhibit a uniform perfusion such as that observed in normal controls. This observation makes colour Doppler analysis a useful parameter, in addition to FSH plasma concentrations, in discriminating between obstructive and non-obstructive azoospermia.

In this study we considered the number of detectable vessels per testis to characterize the blood flow supply of the whole testis according to Bader et al. (1997). In subjects affected by non-obstructive azoospermia, no intrastesticular vessels were detected in either testis of three subjects and in one testis out of two. In the remaining subjects, fewer than three vessels were observed. It has been reported that, in young boys, testicular blood flow depends on testicular volume and that it increases when the maturation process leading to spermatogenesis appears (Bader et al., 1997), suggesting a relationship between blood flow and testicular tubular function. In our patients, testicular volume was reduced but this was not related to a reduction of intratesticular vessels. We postulate that, in these cases, the presence of blood vessels may be related to residual spermatogenetic activity. On the other hand, it is possible that in these regions spermatogenesis could be present because blood vessels survived testicular damage and, therefore, the testicular tissue they supplied survived as well. To verify our hypothesis we performed testicular fine needle aspiration in regions corresponding to detectable vessels, and showed the presence of spermatogenic cells (including spermatids and mature spermatozoa) in 12 out of 16 cases. In these subjects, the previous standard fine needle aspiration of the testis had revealed in all cases the presence of only Sertoli cells. In four testes, cytological analysis showed only Sertoli cells despite the presence of detectable vessels. Interestingly, in these patients, the detectable vessels were localized in the middle portion of the testis corresponding to the mediastinum while in the other cases vessels were present in peripheral regions. The testicular mediastinum is the entry site for vessels, and so the presence of vessels in this region may not be related to spermatogenic areas, and hence be false positive images.

In conclusion, the results of this study demonstrate that colour and power Doppler sonography represent promising methods for the assessment of patients affected by azoospermia allowing us to discriminate obstructive azoospermia (normal vessel distribution) from non-obstructive azoospermia (reduced or absent testicular vessels). Furthermore, the findings of this study, although they cannot be extrapolated to all non-obstructive azoospermic subjects, suggest that the presence of blood vessels, especially in peripheral regions, may indicate the possible presence of residual spermatogenic areas.

References


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