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BRIEF REPORT

Testicular Sertoli cell function in ankylosing spondylitis

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Abstract To assess the testicular Sertoli cell function according to inhibin B levels in ankylosing spondylitis (AS) patients and the possible effect of anti-TNF therapy on this hormone production, 20 consecutive AS patients and 24 healthy controls were evaluated. At study entry, AS patients were not receiving sulfasalazine/methotrexate and never have used biological/cytotoxic agents. They were assessed by serum inhibin B levels, hormone profile, urological examination, testicular ultrasound, seminal parameters, and clinical features. Ten of these patients received anti-TNF treatment and they were reevaluated for Sertoli function and disease parameters at 6 months. Four of them agreed to repeat sperm analysis. At study entry, the median of inhibin B (68 vs. 112.9 pg/mL, $p=0.111$), follicle-stimulating hormone levels (3.45 vs. 3.65 IU/L, $p=0.795$), and the other hormones was comparable in AS patients and controls ($p>0.05$). Sperm analysis was similar in AS patients and controls ($p>0.05$) with one AS patient presenting borderline low inhibin B levels. Further analysis at 6 months of the 10 patients referred for anti-TNF therapy, including one with borderline inhibin B, revealed that median inhibin B levels remained stable (116.5 vs. 126.5 pg/mL, $p=0.431$) with a significant improvement in C-reactive protein (27.8 vs. 2.27 mg/L, $p=0.039$). Sperm motility and concentration were preserved in the four patients who repeated this analysis after TNF blockage. In conclusion, this was the first study to report, using a specific marker, a normal testicular Sertoli cell function in AS patients with mild to moderate disease activity.

Keywords Ankylosing spondylitis · Anti-TNF therapy · Fertility · Inhibin B · Male · Sperm

Introduction

Ankylosing spondylitis (AS) is a chronic inflammatory disease affecting patients during their reproductive years with a significant proportion of male gender [1]. Novel therapeutic approaches have improved quality of life including testicular function and fertility [2].

Of note, inhibin B is an important testicular Sertoli cell function marker since this hormone is a direct product of the seminiferous tubules, allowing a global evaluation of testicular tissue, as previously reported in our male systemic lupus erythematosus patients [3]. This hormone is a heterodimeric glycoprotein hormone produced exclusively by testes' Sertoli cells. It is the male active physiological form of inhibin B in circulation and therefore it is the most important endocrine marker for monitoring the gonadal function in healthy men and in those with testicular dysfunction and seems to be a direct marker of spermatogenesis [4]. This serum fertility marker was reduced in male acute lymphoblastic leukemia survivors treated with chemotherapy [5] and in male systemic lupus erythematosus who received cyclophosphamide [3].

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We recently reported that sperm quality was comparable in AS patients without sulfasalazine/methotrexate and ever use of biological/cytotoxic agents and in healthy controls [6], and a recent study observed more semen alterations in patients with spondyloarthritis before than after TNF blockage [7]. However, there is no systematic analysis of testicular Sertoli cell function in AS patients and controls, particularly pre and post anti-TNF blockage therapy.

Therefore, the aim of this study was to perform testicular Sertoli cell function assessment in male AS patients and to evaluate the possible effect of anti-TNF therapy on inhibin B production.

Materials and methods

AS patients and controls

Twenty AS male postpubertal [8] patients aged between 17 and 53 years regularly followed at the Spondyloarthritis Clinics of the Rheumatology Division, Faculdade de Medicina da Universidade de São Paulo were selected for this study. All patients fulfilled the modified New York criteria for AS diagnosis [9]. At study entry, AS patients were not receiving sulfasalazine/methotrexate for at least 3 months and they never have used biological/cytotoxic agents. There was no restriction to the use of nonsteroidal anti-inflammatory drugs and/or low-dose prednisone (≤10 mg/day). The control group included 24 postpubertal [8] healthy subjects followed at the Urology Division of our university hospital.

Ten of these AS patients received anti-TNF treatment and they were reevaluated for testicular cell Sertoli function and disease parameters at 6 months. Adalimumab (40 mg every 2 weeks) was administered in eight AS patients and etanercept (50 mg weekly) in two. Four of them agreed to repeat sperm analysis at 6 months of anti-TNF blockage therapy.

None of the participants had hydrocele, hypospadia, cryptorchidism, testicular infection, testicular cancer, orchitis, testicular vasculitis, ureteral impairment, previous history of any scrotal or inguinal surgery (e.g., varicocelectomy, vasectomy, and hernia repair), diabetes mellitus, and previous or current history of alcohol or tobacco use. The Local Ethics Committee approved the study and an informed consent was obtained from all participants.

Testicular function evaluation

The testicular function evaluation included the following:

1. Testicular Sertoli cell function: The testicular function was determined by serum inhibin B levels at study entry and 6 months after anti-TNF therapy, blinded to the other parameters of testicular function. This hormone was measured by enzymatically amplified two-site two-step sandwich-type immunoassay. In the assay, duplicated samples were incubated in microtitration wells, which have been coated with anti-inhibin β_B antibody, following the manufacturer's protocol (Diagnostic Systems Laboratories, Inc., Webster, TX, USA) [3]. Intra- and inter-assay coefficients of variation were limited to 3.5–5.6 and 6.2–7.6 %, respectively. Inhibin B normal range was defined as 25–325 pg/mL.
2. Urologic examination: At study entry, a systematic clinical examination of the genitalia was performed in patients and controls, blinded to the other parameters of gonadal function by the same expert urologist and included evaluation of testicles, epididymis, vas deferens, scrotum, and penis [3, 4, 10-13]. Testicles were examined in a warm room with temperature not inferior to 22 °C, in both the standing and supine positions, and with and without Valsalva maneuver [14].
3. Testicular ultrasound: Ultrasound was performed in all AS patients and controls by an expert sonographer using a 14-MHz sector scanner (Logic 9-GE-, Milwaukee, WI, USA) blinded to the other parameters of gonadal function at study entry. Testes were scanned in axial and longitudinal planes, and at least two measurements of length, width, and thickness were obtained and used to calculate the testicular volume. The normal testicular volume value in male postpubertal adolescents and adults is 15±8 mL. The assessment of radiographic varicocele and measurement of pampiniform venous plexus (spermatic plexus) were also evaluated in both testicles [6].
4. Hormonal profile: For the evaluation of the integrity of the hypothalamic–pituitary–gonadal axis function, hormonal measurements were performed at study entry in AS patients and controls, blinded to the other parameters of gonadal function. Abnormal results were repeated for confirmation. Follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin, total testosterone, triiodothyronine (T3), tetraiodothyronine (T4), free T4, and thyrotropin (thyroid-stimulating hormone) levels were detected by fluoroimmunoassay using kits from DELPHIA® time-resolved fluoroimmunoassay (WALLAC Ou, Turku, Finland). Intra- and inter-assay coefficients of variation were limited to 3.5 and 2.1 %, respectively. The normal ranges of hormones were 1–10.5 IU/L for FSH, 1–8.4 IU/L for LH, 2–10 ng/mL for prolactin, and 271–965 ng/dL for morning total testosterone.
5. Semen analysis: Semen analysis was performed according to the guidelines of the World Health Organization (WHO) [15] by two expert medical technologists, blinded to the other parameters of gonadal function. At study

entry, all AS patients and controls were asked to provide two semen samples, collected by masturbation and processed within 1 h of liquefaction, with an interval of 15–30 days after 48–72 h of sexual abstinence. Four of those patients under anti-TNF blockage therapy agreed to repeat sperm analysis at 6 months. The median of the two samples was assessed in regard to sperm concentration, sperm motility, and sperm morphology. The motility of each spermatozoa was graded as "a" (rapid progressive motility), "b" (slow or sluggish progressive motility), "c" (non progressive motility), and "d" (non motility). A patient was considered to have oligozoospermia when a sperm concentration of <20 million/mL was found in the ejaculate. Asthenozoospermia was defined as normal sperm motility (a+b+c) in <50 or <25 % in progressive motility (a+b) within 60 min of ejaculation. Teratozoospermia was defined as normal sperm morphology in <15 % by the WHO criteria [15].

6. Clinical and laboratory evaluation of AS patients: These parameters were assessed at study entry and 6 months after anti-TNF therapy and included the Bath AS Disease Activity Index (BASDAI) [16], erythrocyte sedimentation rate (ESR) by the Westergren method, and C-reactive protein (CRP) by nephelometry.

Statistical analysis

Results were presented as the median (range) or mean ± SD for continuous variables and as the number (in percent) for categorical variables. Data were compared by t tests or by the Mann–Whitney test for continuous variables to evaluate differences between AS patients and controls and between AS patients before and after anti-TNF therapy. For categorical variables, differences were assessed by Fisher's exact test. p values less than 0.05 were considered significant.

Results

AS patients vs. controls

At study entry, the median of current and spermarche age were similar in AS patients and controls [33 (17–53) vs. 28.5 (15–54)years, p=0.175; 13 (9–18) vs. 12 (11–15) years, p=0.358, respectively].

The median of inhibin B levels was comparable in AS patients and controls [68 (23–265) vs. 112.9 (47.8–231.9) pg/mL, p=0.111]. Only one (5 %) AS patient had borderline low inhibin B levels (23 pg/mL) compared to none of controls (p=0.454). The median of FSH levels [3.45 (1.4–10.7) vs. 3.65 (1.8–5.8)IU/L, p=0.795] and the other hormones was also similar in both groups (p>0.05). No differences were evidenced in the frequency of elevated

Table 1 Demographic data, testicular function, and disease activity parameters in 10 patients with ankylosing spondylitis before and after 6 months of anti-TNF therapy

Patients	Pre anti-TNF								Post anti-TNF					
	Current age (years)	Disease duration (years)	Inhibin B (pg/mL)	Sperm analysis	Varicocele	ESR (mm/first hour)	CRP (mg/L)	BASDAI	Biological agents	Inhibin B (pg/mL)	Sperm analysis	ESR (mm/first hour)	CRP (mg/L)	BASDAI
1	32	10	134	Terato	+	10	39.3	6.0	ADA	316	Normal	6	1.83	2,62
2	43	30	265	Normal	+	6	3.24	4.11	ADA	255	ND	26	1.02	3,24
3	30	13	117	Normal	+	48	30.6	4.68	ADA	142	ND	7	3.29	2.3
4	29	3	115	Terato	+	34	60.2	5.58	ADA	126	Terato	2	1.45	1.0
5	34	12	116	Oligo/terato	+	10	9.59	4.32	ADA	127	ND	1	0.38	1.32
6	24	10	159	Terato	–	11	8.83	6.08	ETA	166	Terato	5	2.71	5.2
7	20	27	132	Oligo/terato	–	26	25	3.14	ADA	125	Normal	2	1.36	0.6
8	49	30	104	Terato	–	33	30.8	2.75	ADA	98	ND	76	46.0	6.3
9	35	9	70	Terato	–	8	38.8	8.68	ADA	54	ND	7	12.4	8.7
10	49	18	28	Normal	–	19	12.1	4.67	ETA	24	ND	29	19.1	8.8

Terato teratozoospermia (abnormal sperm morphology), *Oligo* oligozoospermia (low sperm concentration), *ESR* erythrocyte sedimentation rate, *CRP* C-reactive protein, *BASDAI* Bath AS Disease Activity Index, *ADA* adalimumab, *ETA* etanercept, *ND* not done

FSH (5 vs. 0 %, p=0.454), elevated LH (0 vs. 4 %, p=1.0), elevated prolactin (5 vs. 4 %, p=1.0), and reduced morning total testosterone levels (5 vs. 4 %, p=1.0) in AS patients and controls. All AS patients and controls had normal thyroid hormones.

All AS patients and controls had normal epididymis, vas deferens, scrotum, and penis. All eight patients with varicocele evaluated in the present study had normal inhibin B levels and comparable to those without this clinical complication [115.5 (54–265) vs. 65 (23–159)pg/mL, p=0.217]. Regarding sperm analysis in AS patients with varicocele, isolated teratozoospermia was observed in four, normal sperm evaluation in three, and terato/oligozoospermia in one. The mean of right and left testicular volumes by testicular ultrasound was similar in AS patients and controls (12.7±3.4 vs. 12.9±3.7 mL, p=0.855; 12.3±3.7 vs. 12.5±3.7 mL, p=0.926, respectively).

Regarding sperm analysis, oligozoospermia (10 vs. 0 %, p=0.201), asthenozoospermia (10 vs. 0 %, p=0.201), and teratozoospermia (45 vs. 28 %, p=0.352) were comparable in AS patients and controls.

AS patients after 6 months of anti-TNF therapy

Further analysis of those 10 AS patients referred for anti-TNF therapy revealed that median inhibin B levels remained stable after 6 months of this treatment [116.5 (28–265) vs. 126.5 (24–316)pg/mL, p=0.431]. Only one AS patient had borderline low inhibin B levels (24 pg/mL) after 6 months of anti-TNF blockage therapy.

Demographic data, testicular function, and disease activity parameters in 10 patients with AS before and after 6 months of anti-TNF therapy are shown in Table 1. Sperm motility and concentration were normal in the four patients who agreed to repeat this analysis after anti-TNF treatment, including one with previous oligo/teratozoospermia. Isolated teratozoospermia was observed in three patients before anti-TNF blockage and in two after this therapy (Table 1).

The median of CRP was significantly higher in 10 AS patients before anti-TNF therapy compared to after 6 months of this treatment [27.8 (3.24–60.2) vs. 2.27 (0.38–46)mg/L, p=0.039]. ESR values [15 (6–48) vs. 6.5 (1–76)mm/first hour, p=0.589] and BASDAI [4.675 (2.75–8.68) vs. 2.93 (0.6–8.8), p=0.3] were also lower but did not reach statistical significance.

Discussion

We identified that inhibin B production is preserved in AS patient even in patients under TNF blockage. The advantage of the present study was the prospective design of a global parameter of testicular function and the inclusion of only postpubertal subjects, assuring a more homogeneous group regarding gonadal evaluation [3]. The rigorous exclusion criteria of AS patients and controls without comorbidities is relevant since diabetes, alcohol intake, and smoking habit may affect testicular function [2]. The low number of treated AS patients with anti-TNF therapy was a limitation of this study. Likewise, the impact of disease vs. therapy on the abnormalities observed was difficult to distinguish, because of a lack of a disease control group.

Sertoli cells are considered as “nurse cells” and they are essential for germ cell development (spermatogonia, spermatocyte, spermatids, and spermatozoa) [17]. Inhibin B is produced by testicular Sertoli cell, and it is considered a marker of fertility and predicts the level of spermatogenesis in adult men [18]. Furthermore, inhibin B and activins regulate FSH secretion, spermatogenesis, and testicular steroidogenesis. In addition, activin A is a paracrine regulator of germ and Sertoli cell proliferation and differentiation [19].

Of note, the present result is restricted to patients with mild to moderate disease activity at study entry and may not be extended to those with severe inflammation, a parameter known to reduce sexual activity and reproduction with downregulation of the hypothalamic–pituitary–gonadal axis [20].

All of patients with varicocele evaluated in the present study had normal inhibin B, indicating that this isolated mild sperm abnormality (teratozoospermia) did not seem to contribute to infertility in AS patient; however, a prospective study is required. Indeed, a recent meta-analysis observed that isolated teratozoospermia was not associated with decreased probability of pregnancy in assisted reproduction [21].

Our data also confirm preserved integrity of Sertoli cell function in AS patients after anti-TNF therapy. Interestingly, TNF was reported to have an effect on germ cell apoptosis, regulation of secretion of peritubular cells, and sperm survival [22]. TNF may also induce alteration of blood–testis barrier permeability with testicular Sertoli cell dysfunction and infertility [23].

In conclusion, this was the first study to report, using a specific marker, a normal testicular Sertoli cell function, unimpaired spermatogenesis, and normal fertility in AS patients with mild to moderate disease activity.

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Disclosures None.

References

1. Feldtkeller E, Khan MA, Van Der Heijde D, Van Der Linden S, Braun J (2003) Age at disease onset and diagnosis delay in HLA-B27 negative vs. positive patients with ankylosing spondylitis. Rheumatol 23:61–66
2. Silva CA, Bonfa E, Ostensen M (2010) Maintenance of fertility in patients with rheumatic diseases needing anti-inflammatory and immunosuppressive drugs. Arthritis Care Res (Hoboken) 62:1682–1690
3. Suehiro RM, Borba EF, Bonfa E, Okay TS, Cocuzza M, Soares PM et al (2008) Testicular Sertoli cell function in male systemic lupus erythematosus. Rheumatology (Oxford) 47:1692–1697
4. Anawalt BD, Bebb RA, Matsumoto AM, Groome NP, Illingworth PJ, McNeilly AS et al (1996) Serum inhibin B levels reflect Sertoli cell function in normal men and men with testicular dysfunction. J Clin Endocrinol Metab 81:3341–5
5. Jahnukainen K, Heikkinen R, Henriksson M, Cooper TG, Puukko-Viertomies LR, Mäkitie O (2011) Semen quality and fertility in adult long-term survivors of childhood acute lymphoblastic leukemia. Fertil Steril 96:837–42
6. Nukumizu LA, Saad GC, Ostensen M, Almeida BP, Cocuzza M, Gonçalves C et al (2012) Gonadal function in male patients with ankylosing spondylitis. Scand J Rheumatol 41(6):476–81
7. Villiger PM, Caliezi G, Cottin V, Förger F, Senn A, Østensen M (2010) Effects of TNF antagonists on sperm characteristics in patients with spondyloarthritis. Ann Rheum Dis 69:1842–4
8. Tanner JM (1962) Growth at adolescence, 2nd edn. Blackwell Scientific Publications, Oxford
9. Van Der Linden S, Valkenburg HA, Cats A (1984) Evaluation of diagnostic criteria for ankylosing spondylitis: a proposal for modification of the New York criteria. ArthritisRheum 27:361–368
10. Soares PM, Borba EF, Bonfa E, Hallak J, Al C, Silva CA (2007) Gonad evaluation in male systemic lupus erythematosus. ArthritisRheum 56:2352–2361
11. Moraes AJ, Pereira RM, Cocuzza M, Casemiro R, Saito O, Silva CA (2008) Minor sperm abnormalities in young male post-pubertal patients with juvenile dermatomyositis. Braz J Med Biol Res 41:1142–1147
12. Moraes AJ, Pereira RM, Cocuzza M, Casemiro R, Saito O, Silva CA (2010) Gonad evaluation in male dermatomyositis. A pilot study. Clin Exp Rheumatol 28:441–442
13. Vecchi A, Borba E, Bonfá E, Cocuzza M, Pieri P, Kim CA et al (2011) Penile anthropometry in systemic lupus erythematosus patients. Lupus 20:512–518
14. Bong GW, Koo HP (2004) The adolescent varicocele: to treat or not treat. Urol Clin N Am 31:509–15
15. World Health Organization (WHO) (1999) Laboratory manual for the examination of human semen and sperm-cervical mucus interaction, 4th edn. Cambridge University Press, New York, pp 1–128
16. Garrett S, Jenkinson T, Kennedy G, Whitelock H, Gaisford P, Calin A (1994) A new approach to defining disease status in ankylosing spondylitis: the Bath Ankylosing Spondylitis Disease Activity Index. J Rheumatol 21:2286–2291
17. Griswold MD (1995) Interactions between germ cells and Sertoli cells in the testis. Biol Reprod 52:211–6
18. McNeilly AS (2012) Diagnostic applications for inhibin and activins. Mol Cell Endocrinol 359:121–5
19. Hedger MP, Winnall WR (2012) Regulation of activin and inhibin in the adult testis and the evidence for functional roles in spermatogenesis and immunoregulation. Mol Cell Endocrinol 359:3042
20. Straub RH, Cutolo M, Buttgereit F, Pongratz G (2010) Energy regulation and neuroendocrine-immune control in chronic inflammatory diseases. J Intern Med 267:543–60
21. Hotaling JM, Smith JF, Rosen M, Muller CH, Walsh TJ (2011) The relationship between isolated teratozoospermia and clinical pregnancy after in vitro fertilization with or without intracytoplasmic sperm injection: a systematic review and meta-analysis. Fertil Steril 95:1141–5
22. Suominen JS, Wang Y, Kaipia A, Toppari J (2004) Tumor necrosis factor-alpha (TNF-alpha) promotes cell survival during spermatogenesis, and this effect can be blocked by infliximab, a TNF-alpha antagonist. Eur J Endocrinol 151:629–40
23. Silva CA, Cocuzza M, Borba EF, Bonfá E (2012) Cutting-edge issues in autoimmune orchitis. Clin Rev Allergy Immunol 42:256–63