Testicular histology may predict the successful sperm retrieval in patients with non-obstructive azoospermia undergoing conventional TESE: a diagnostic accuracy study

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Abstract

Purpose The present study sought to determine the diagnostic accuracy of FSH level, testicular volume, and testicular histology in predicting the successful sperm retrieval (SSR) in a large cohort of patients with non-obstructive azoospermia undergoing conventional testicular sperm extraction (TESE).

Methods We retrospectively evaluated 356 patients with non-obstructive azoospermia between June 2004 and July 2009. Binary logistic regression was used to evaluate the diagnostic accuracy of our predicting model, identifying sperm retrieval rate as binary dependent variable. The predictive accuracy of all variables individually evaluated was quantified with area under curve (AUC) estimates derived from receiver operating characteristic (ROC) curve.

Capsule This study demonstrates that including testicular histology in a model for predicting sperm retrieval increases its diagnostic accuracy.

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Results The mean patients' age was 36.8 years. Testicular sperm were retrieved in 158 out of 356 patients (44.3 %). Histological diagnosis of Sertoli cell only syndrome (SCO) was obtained in 216 patients (60.6 %), while 55 patients (15.4 %) had maturation arrest (MA) and 85 (23.8 %) had hypospermatogenesis (HYPO). The binary logistic regression model was statistically significant ($\chi^2 = 96.792$, $p < 0.0001$) and correctly classified 72.8 % of cases with 46.8 % sensitivity and 93.4 % specificity, positive predictive value (PPV) 85.06 %, negative predictive value (NPV) 68.7 %, +likelihood ratio (LR) 7.13, and -LR 0.57. Only testicular histology was significant to the model, while FSH and testicular volume were not. Sperm retrieval rate (SRR) was significantly higher in patients with HYPO compared to patients with SCO or MA (88.2 vs 30.5 and 30.9 %, respectively, $p < 0.0001$).

Conclusions This study demonstrates that including testicular histology in a model for predicting sperm retrieval increases its diagnostic accuracy. As histology is not available prior to TESE, this model applies only to patients with previous testicular surgery.

Keywords Non-obstructive azoospermia · Testicular sperm extraction · Sperm retrieval · FSH · Testis histology · Testis volume

Introduction

Azoospermia, defined as the absence of spermatozoa in the ejaculate after assessment of centrifuged semen on at least two occasions, is a non-rare condition, as it can be diagnosed in 1 % of the male population and in up to 15 % of the infertile male subjects [1]. Once thought to be sterile with the sole chance to father a child being dependent on donor insemination, azoospermic patients are now given the opportunity of using in vitro fertilization techniques, namely intracytoplasmic sperm injection (ICSI), with sperm retrieved from their own testes. As the reproductive ability

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of azoospermic patients entering an ICSI cycle has been demonstrated to be roughly comparable to that of oligozoospermic subjects [2], finding viable sperm in the patients' testes represents the most relevant step before treatment. While sperm retrieval rates (SRR) for men with obstructive azoospermia are excellent (96–100 %), the reported overall SRR for patients with non-obstructive azoospermia (NOA) ranges from 30 to 60 % [3]; therefore, identifying those clinical parameters that may reliably predict the chance of retrieving viable sperm in individual NOA patients would be crucial for assisting surgeons and clinicians in the correct counseling of such patients.

Studies in the field seem to demonstrate that sperm retrieval in patients with NOA is quite an unpredictable factor, given that FSH, testicular volume, and other clinical characteristics have low sensitivity and specificity when their predictive ability is individually evaluated [4]. More recently, a couple of studies modeled a combination of some preoperative clinical parameters in the effort of achieving a higher diagnostic accuracy: Boitrelle et al. [5] calculated a score from the combination of serum FSH and inhibin B levels plus testicular volume in patients undergoing conventional testicular sperm extraction (cTESE) obtaining an area under curve (AUC) value of 0.66, while Ramasamy and coworkers used an artificial neural network and were able to identify patients with or without testicular spermatozoa retrieved by microdissection testicular sperm extraction (microTESE) with a 60.8 % diagnostic accuracy [6]. Both models did not take into account testicular histology as potential predictive factor for sperm retrieval success, as they considered only pre-clinical, non invasive parameters.

Notably, testis histology has been found to be the most reliable predictive factor of successful sperm retrieval (SSR) in NOA patients since the late nineties [7]. Although diagnostic testicular biopsy is no longer considered as pre-clinical tool for patients with NOA [8], it is not uncommon that patients with previous testicular surgery and unsuccessful sperm retrieval could consider attempting another surgical treatment aimed at retrieving testicular sperm. In these cases, it has been proposed that the previously obtained testicular histology could help in predicting the sperm retrieval success in a further surgical attempt [9–13]

In the effort of increasing the diagnostic accuracy of the pre-surgical evaluation of patients with NOA, we sought to determine the predictive potential of the combined evaluation of FSH level, testicular volume, and testicular histology on the chance of achieving a SSR in these patients.

Materials and methods

Patients

We retrospectively collected data from the records of azoospermic patients referring to our center to undergo

testicular sperm retrieval aimed to ICSI from June 2004 to July 2009. Patients received the diagnosis of non-obstructive azoospermia when the absence of spermatozoa in at least two semen analyses with centrifugation and careful examination of pellet was recorded, ejaculate volume was higher than 1.0 ml, and pH was higher than 7.2. Patients with normal FSH and testicular volume underwent also scrotal and transrectal ultrasound in order to exclude the presence of epididymis head and/or tail dilation, bilateral absence of vas deferens, ejaculatory duct abnormalities, prostate median cysts or seminal vesicles enlargement, or emptying impairment suggestive of obstruction of the male genital tract [14].

Exclusion criteria were obstructive azoospermia, known cytogenetic abnormalities, Y chromosome microdeletions, and testosterone level lower than 300 ng/dl. Only patients who gave their informed consent to the surgical procedure and to the anonymous utilization of their clinical data were included in the study.

The week before surgery, all underwent a careful evaluation of the volume of each testis by ultrasonography (volume (ml) = $0.52 \times A \times B \times C$, A, B, and C being the measurement of the three testicular axes as expressed in cm). The mean bilateral testicular volume was then calculated. Blood samples were also obtained to assay patients' FSH serum level. An additional semen sample was obtained on the day of planned surgical procedure in order to confirm the absence of sperm in the pellet.

Surgical procedure and sample processing

TESE was performed by removing a large fragment of testicular tissue of about $8 \times 4 \times 3$ mm through a transversal incision of the albuginea, either equatorially or in the cranial part of the testis. The fragments were washed in human tubal fluid medium to remove the blood, and given to the biologist for the microscopic examination. Only afterwards, the testicular tissue surfaces were irrigated for antiseptis with Ringer solution added with 80 mg gentamicin/100 ml. Hemostasis was then performed by gently pressing the testicular tissue for 2 min using gauze wet with the above antiseptic solution followed by very limited and careful bipolar microcoagulation. The tunica vaginalis opening was repaired by a continuous vicryl 5/0 or 4/0 after instillation into the vaginalis cavity of 1.5 mg betamethasone to prevent pain and tunica vaginalis adhesions.

Each testicular tissue fragment was placed in sterile Petri dishes with 0.5 ml of a sperm washing medium and dispersed by isolating individual seminiferous tubules with glass slides and mincing individual tubules. Each tissue sample was used to quantify the presence and number of spermatozoa present. When the fluid obtained after the suspension's centrifugation showed more than 100 spermatozoa/mm³, the viability of the retrieved spermatozoa was assessed by eosin–nigrosin live/dead stain test. The sperm retrieval was considered to be

successful when the number of viable sperm retrieved was at least equivalent to the number of oocytes retrieved in the female partner.

Testicular histology

Due to an agreement between our unit and the pathology department of the same hospital, the histological evaluation of all testes samples was performed by the same pathologist (GG) with more than 10 years of experience in the field. A fragment of subcapsular parenchyma was removed soon after the incision of the albuginea, then fixed in Bouin's solution, and sent to the pathologist. Histological analysis was conducted by examining at least 100 different sections of tubuli seminiferi. Histopathologic evaluation included also any abnormal features such as the presence of testicular intraepithelial neoplasia. The histological results were defined as follows: (i) Sertoli cell only syndrome (SCO) when the tubules were prevalently or exclusively populated by only Sertoli cells, (ii) late maturation arrest (MA) characterized with an arrest of the spermatogenetic maturation sequence at the level of round spermatid, and (iii) hypospermatogenesis (HYPO) in which tubules showed a severely reduced population of germ cells; all stages of germ cells (spermatogonia, spermatocytes, and spermatids) were present but reduced in number.

Statistical analysis

The difference in serum FSH level, testicular size, and testicular histology in patients stratified according to SSR was evaluated by Mann–Whitney *U* test and the *z*-score test for two population proportions.

Prediction of SSR using the combination of FSH level, testicular volume, and testicular histology was performed by binary logistic regression, identifying SSR as binary dependent variable, FSH and testicular volume as continuous variables, and testicular histology as categorical (nominal) variable utilizing contrast coding. The predictive accuracy of all the variables individually evaluated was quantified with AUC

estimates derived from receiver operating characteristic (ROC) curve.

FSH level and testicular size were also evaluated by Kruskal–Wallis test using testicular histology as grouping variable. SSR in patients stratified by testicular histology was computed by chi-square (χ^2) analysis on the basis of a 3×2 contingency table.

Statistical significance was set at $p < 0.05$ for all analyses. All computations were performed using SPSS for Windows.

Power calculation

The sample size requested for binary logistic regression was calculated according to Peduzzi and coworkers [15]. Given that the proportion of positive cases (p) was 44.3 % (0.426) and the number of independent variables (k = FSH, testis volume, and histology) was three, the minimum number of cases to include in this study to obtain a statistical power of 80 % with α set at 0.05 was ($N = 10 k/p$) 68.

Results

The mean patients' age was 36.8 years (18–63 years). Successful sperm retrieval was obtained in 158 out of 356 patients (44.3 %). Mean FSH serum level was 19.6 mIU/ml (95 % CI 1.54, range 0.6–127), while the mean testicular volume was 7.9 ml (95 % CI 0.41, range 0.89–20). Histological diagnosis of SCO was obtained in 216 patients (60.6 %), while 55 patients (15.4 %) had MA and 85 (23.8) had HYPO.

When patients' characteristics were evaluated according to sperm retrieval success (Table 1), patients with SSR were found to have significantly lower serum FSH level and higher testicular volume compared to patients with sperm retrieval failure. However, when the predictive accuracy of both variables was individually quantified with ROC curve AUC estimates, the ability of the test to correctly classify patients with and without SSR was insignificant (AUC 0.36) and poor (AUC 0.61) for FSH and testicular volume, respectively.

Table 1 Comparison of clinical parameters in patients stratified according to sperm retrieval success

Parameter	Successful sperm retrieval	No sperm retrieved	<i>p</i> value
Patients	158	198	
FSH mIU/ml mean (95 % CI) [range]	16.1 (1.79) [0.6–58]	22.4 (2.32) [1.1–127]	<0.0001 ^a
Testes volume ml mean (95 % CI) [range]	8.7 (0.63) [1.4–20]	7.2 (0.52) [0.8–19]	<0.0001 ^a
SCO <i>N</i> (%)	66 (41.8)	150 (75.8)	<0.0001 ^b
MA <i>N</i> (%)	17 (10.8)	38 (19.2)	<0.0001 ^b
HYPO <i>N</i> (%)	75 (47.4)	10 (5)	<0.0001 ^b

^a Mann–Whitney *U* test

^b *Z*-score for two population proportions

Table 2 Binary logistic regression of predictors of sperm retrieval

	OR (95 % CI)	<i>p</i> value
FSH	0.98 (0.96–1.008)	0.25
Testicular volume	1.063 (0.994–1.13)	0.073
Testicular histology	0.071 (0.034–0.14)	<0.0001

The best cutoff value for testicular volume was 6.57 ml, with a sensitivity of 68.4 % and a specificity of 49.5 %. ROC curve was not run for testicular histology being this a non continuous variable.

We then performed a binary logistic regression model to ascertain the predictive value of patients' FSH, testicular volume, and testicular histology on the likelihood of retrieving testicular sperm. The model was statistically significant ($\chi^2 = 96.792$, $p < 0.0001$) and correctly classified 72.8 % of cases with 46.8 % sensitivity (95 % CI 38.86–54.93) and 93.4 % specificity (95 % CI 89.03–96.4), positive predictive value (PPV) 85.6 %, negative predictive value (NPV) 68.7 %, +likelihood ratio (LR) 7.13 (95 % CI 4.11–12.38), and –LR 0.57 (95 % CI 0.49–0.66). Only testicular histology was significant to the model, while FSH and testicular volume were not (Table 2).

To confirm the results of the binary logistic regression, we looked at patients' characteristics as stratified according to their testicular histological pattern (Table 3). SRR was significantly higher in patients with HYPO compared to patients with SCO or MA (88.2 vs 30.5 and 30.9 %, respectively, $p < 0.0001$). Patients with SCO had significantly higher FSH level and lower testicular volume compared to patients with MA and HYPO.

Discussion

The results of the present study demonstrate that including the pattern of testicular histology in a model for predicting SSR in patients with NOA improves its diagnostic accuracy compared to the predictive models employed to date. Notably, no previous studies included testis histology as independent variable in a model for the prediction of SSR in NOA patients prior to cTESE.

Our intention was to give a contribution to the debate on the ability of clinical characteristics on selecting the ideal candidates to cTESE among patients with NOA. In order to avoid confounding factors, we excluded patients with hypotestosteronemia, given that studies demonstrated that optimizing testosterone level may improve spermatogenesis [16] and increase SRR [17]. We also excluded patients with Y microdeletions, given that this clinical condition has been found to be an independent predictor factor for SSR [11].

Our data are consistent to those of studies evaluating large cohorts of patients demonstrating that serum FSH level and testicular volume are not predictive of SSR when evaluated as the unique independent predictive factor [6, 18, 19]. The poor relationship between FSH level and spermatogenesis can be explained because FSH secretion and release is controlled by too many endocrine and paracrine factors that ultimately contribute to its serum levels, without ignoring the contribution of the polymorphisms in FSHB genotype and FSHR genotype that may lead to higher than expected FSH levels in some patients [20], so that a linear relationship between FSH serum level and spermatogenesis cannot be expected in all patients. On the other hand, the poor predictive role of testicular volume can be explained by the presence of focal area of intact spermatogenesis that may be found even in patients with testicular atrophy [18, 21]. Our data demonstrate that if we would rely on testicular volume to obtain indication for selecting the optimal candidates to surgical sperm retrieval, we would wrongly include and exclude from attempting surgery 32 and 50 % of patients, respectively.

On the other hand, the present study demonstrated that testicular histology may be a good predictive factor for SSR. The binary logistic regression analysis provided an OR ratio below 1 because histology was computed as categorical variable utilizing HYPO as reference level in contrast coding with SSR as binary dependent value. As a result, when patients' histology differed from HYPO, the chance of SSR decreased, in accordance with the findings of a recent study [22]. Notably, according to the value of +LR ratio, patients with histology different from HYPO were seven times more likely to fail the sperm retrieval procedure.

The reliability of testicular histology as predictor of SSR has been suggested by several studies, as mentioned in the “Introduction” section, but its application in the clinical

Table 3 Comparison of clinical characteristics in patients stratified according to histology

	SCO	MA	HYPO	<i>p</i> value
FSH mIU/ml mean (95 % CI) [range]	24.3 (2.13) [1.1–127]	11.8 (2.38) [0.6–33.6]	12.7 (2.05) [2.45–56]	<0.0001 ^a
Testes volume ml mean (95 % CI) [range]	7.09 (0.47) [0.89–20]	9.41 (1.16) [1.42–18]	9.1 (0.87) [2.88–20]	<0.0001 ^a
Sperm retrieval rate %	30.5	30.9	88.2	<0.0001 ^b

^a Kruskal–Wallis test

^b Chi square analysis on the basis of 3 × 2 contingency table

practice has been questioned due to some criticisms. The major and unquestionable criticism was that histology may be applicable only to those patients with previous testicular surgery, since a diagnostic testicular biopsy is not cost-effective being an invasive procedure not free from complications.

Relevant to this point, not few NOA patients with unsuccessful sperm retrieval may undergo another or more surgical attempts before turning to donor insemination. Since obtaining testicular samples for histology during testicular surgery may be useful for detection of carcinoma in situ of the testis, which occurs in 1–5 % of infertile men [23], it is not uncommon for patients with previous failed cTESE to present clinical dossiers that include reports on previously obtained testicular histology. Such reports could provide valuable information to assist the urologist in choosing the more appropriate surgical technique of sperm retrieval for another surgical attempt. As suggested by the results of the latest systematic reviews [24, 25], microTESE may perform better in patients with SCO or MA, while in patients with hypospermatogenesis, TESE and microTESE yield comparable sperm retrieval rates. Consequently, among patients with sperm retrieval failure, those with histology of HYPO could be suggested to undergo another TESE attempt rather than considering to undergo the more invasive microTESE. Our data provide an additional and substantial (due to our large sample size) demonstration for the high chances of retrieving testicular sperm by conventional TESE in patients with NOA with histology of HYPO.

It is to be remarked that, in order to obtain a reliable histological picture, the pathologist needs to be provided with tissue properly fixed so that the cell types during spermatogenesis can be easily recognized, and an in-depth experience in the field is required (McLachlan et al. 2007). One strength of the present study is that the histological evaluation of all the testicular samples was performed by the same well-experienced pathologist.

An obvious limitation of this study is its retrospective design, but no data were missing, as all clinical data were carefully stored both in the hospital archive and in an electronic datasheet. We are also aware that study evaluating predictive factors for SSR in NOA patients undergoing cTESE may sound old-fashioned, given that microTESE is being considered the treatment of choice for patients with NOA and may be successful in these patients regardless of any clinical characteristic. Nevertheless, it has to be pointed out that such surgical technique is not feasible in all IVF centers as it requires microsurgical equipment such as operating microscopes, must be performed by well-trained urologists, necessitates general anesthesia, and is associated with increased operative time and costs; on the other hand, our results suggest that cTESE may be a cost-effective treatment in NOA patients with testicular histology of HYPO; therefore, the latter patients may undergo a

less invasive treatment with unaltered chances of having their sperm retrieved.

In conclusion, this study demonstrates that including testicular histology in a model for predicting sperm retrieval significantly increases its diagnostic accuracy. As testicular histology can be obtained only after testicular surgery, this model may fit well to NOA patients with previous sperm retrieval failures.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Funding source No funds were requested for this study.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

For this type of study formal consent is not required.

IRB approval IRB approval was requested to the local ethical committee.

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