The Prediction of Sperm Chromatin Structure Assay (SCSA) Parameters for Outcomes of Intrauterine Insemination

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Abstract

Background: Many studies have assessed the association between sperm DNA fragmentation and outcomes of ART. But the published papers have not offered enough evidence about whether sperm DNA fragmentation tests could make suggestions to predict intrauterine insemination (IUI) outcomes. The aim of this study was to assess whether the sperm chromatin structure assay (SCSA) parameters, sperm DNA fragmentation index (DFI) and high DNA stainability (HDS), could be used as predictors for treatment outcomes in IUI program.

Methods: A retrospective cohort study was conducted at a large reproductive medicine center. 1139 IUI cycles from 1139 couples were studied. The association of SCSA parameters with the clinical pregnancy and early pregnancy loss after IUI were analyzed.

Results: Clinical pregnancy rate per cycle in DFI<15%, 15%≤DFI<30%, and DFI≥30% groups were 11.1%, 7.3%, and 8.9%, respectively, with no statistical differences between the groups (P=0.127). Pregnancy loss rate were 24.3%, 27.6%, and 14.3%, respectively, with no statistical differences (P=0.762). Clinical pregnancy rate per cycle in HDS≤15% and HDS>15% groups were 9.8% and 7.5%, respectively, with no significant difference (P=0.468), and the pregnancy loss rate were 26.2% and 0, respectively, and also no statistical difference (P=0.191). Multivariate logistic regression analysis showed a higher rate of clinical pregnancy in couples with a younger female (OR=0.90, 95% CI: 0.83-0.97, P=0.007), and in couples with a male who had higher sperm concentration after washing (OR=1.02, 95% CI: 1.00-1.04, P=0.035). A higher risk of pregnancy loss was observed with increased female age (OR= 1.43, 95% CI:1.09-1.89, P=0.010) and lower sperm concentration after washing (OR= 0.92, 95% CI: 0.84-0.99, P= 0.029).

Conclusions: Sperm DFI and HDS were not significantly correlated with clinical pregnancy and pregnancy loss in cycles of IUI. Female age and motile sperm concentration had statistically significant effects on both clinical pregnancy and pregnancy loss after IUI treatment.

Background

Intrauterine insemination (IUI) is the first line treatment for couples with unexplained or mild male-factor infertility. Compared with IVF/ICSI treatment, IUI is simple, easy to manage, and has a relatively low cost. The clinical pregnancy rates of IUI per cycle is between 5%-13% [1–3]

The outcomes of IUI vary widely, correlated with factors such as female age, endometrial thickness, follicles maturity, aetiology of infertility, protocols of ovarian stimulation, timing and number of inseminations and characters of sperm[4]. Besides female factors, sperm quality is a major influencer of successful pregnancy. Sometimes even with normal traditional semen parameters, subfertile men may have abnormal sperm DNA/chromatin integrity[5]. Measuring two parameters sperm DNA fragmentation index (DFI) and high DNA stainability (HDS), the sperm chromatin structure assay (SCSA®) is able to reveal the degree of sperm DNA damage and the abnormal lack of sperm chromatin condensation respectively[6].
Numerous published studies have focused on the association between sperm DNA fragmentation and IVF/ICSI treatment outcomes, but the results have been inconsistent and controversial [7]. As such, more and more research concerning this topic has been performed, especially in couples with unexplained infertility. However, research on the relationship between sperm DNA fragmentation and IUI outcomes is comparatively less. Up to now, the published papers have not offered enough evidence about whether sperm DNA fragmentation tests could be used to predict IUI outcomes or to make suggestions for the use of more invasive approaches.

Therefore, our study was to understand the value of the SCSA test in IUI outcomes, and to provide more information to researchers worldwide. We aimed to test whether SCSA parameters, DFI and HDS, could be used as independent predictors of IUI outcomes and to investigate which factors might affect the clinical pregnancy and early pregnancy loss in IUI programs.

**Methods**

This was a retrospective study performed at the Center of Reproductive Medicine of Peking University Third Hospital. Data were retrieved from our clinical database and SCSA tests were carried at the Andrology Laboratory. The study was approved by the local Ethics Committee.

**Patients**

Couples who underwent IUI from January 2017 to December 2019 were chosen. The couples had to have a child wish for 1 year, have at least one patent fallopian tube (as checked by hysterosalpingography or by laparoscopy), and have an ovulatory cycle. Women with and without ovarian stimulation were included. Couples with a male who had never taken the SCSA test were excluded. Only the first treatment cycle of the chosen couples during this period (January 2017 to December 2019) was included.

**SCSA® Test Protocol**

The SCSA was performed according to the manufacturer’s instruction, and the SCSA kit was purchased from CellPro Biotech Co., Ltd. (Ningbo, China). An aliquot of raw semen was transferred to buffer A (0.01 M Tris–HCl, 0.15 M NaCl, 1 mM disodium EDTA, pH 7.4) at 4°C to a final sperm concentration of $1 \times 10^6$ sperm/mL, and a final volume of 100µL. Samples were added to 200µL of a detergent solution B (0.1% Triton X-100, 0.15 M NaCl, 0.08 M HCl, pH 1.2) for 30 s. After 30 s, 600µL of staining buffer C (6 µg/mL AO, 37 mM citric acid, 126 mM Na$_2$HPO$_4$, 1 mM disodium EDTA, 0.15 M NaCl, pH 6.0) was added to the test tube and mixed. After 3 minutes of incubation, the sample was placed into the BD FACS Calibur™ flow cytometer (Becton Dickinson, San Jose, CA). Then the sperm DFI and HDS were calculated through fluorescence signals. At least 5000 cells were recorded and analyzed with data analysis software (DFIView 2010 Alpha 11.15, CellPro Biotech, Ningbo, China) for double-stranded (green: native) and single-stranded (red: damage) DNA. The proportion of the sperms with red fluorescence by the total sperm number was calculated, namely DFI. And HDS was obtained by the fraction of sperms with a higher level of green fluorescence. As a quality control, a sample with known DFI and HDS was
evaluated each day of analyses. The DFI was categorized as low (DFI ≤ 15%), medium (15%–15%) was marked to suggest that sperm mature chromatin content was abnormal.

**IUI Program**

In addition to natural cycles, cycles with ovarian stimulation used three types of protocols (clomiphene citrate, letrozole and HMG). Follicle growth was monitored with transvaginal ultrasound by gynecologists in the reproductive medicine center. In both natural and stimulated cycles, follicle development was monitored from day 8–10 of the menstrual cycle, and then rechecked every 2 or 3 days depending on the follicle sizes. If 1–2 follicles reached 18 mm of diameter, 5,000–10,000 IU urinary human chorionic gonadotropin (hCG) (Livzon®, Zhuhai, China) was injected intramuscularly to trigger ovulation. Insemination was performed 36–40 hours after hCG injection. On this day, semen was collected at the laboratory after 3–7 days of abstinence and prepared with swimming-up after liquefaction. The count and motility of spermatozoa were evaluated. Then 0.8 mL volume of washed semen sample was used for insemination. After insemination, women had bedrest for 30 minutes. From the second day after insemination, luteal phase support was applied to all women.

**Outcomes**

Serum hCG was collected to detect pregnancy on the 16th day after insemination. If the result was positive, an ultrasound was performed after 16–19 days. Clinical pregnancy was confirmed by the sonographic evidence of an intrauterine gestational sac. Loss of the fetus within 12 weeks of pregnancy was classified as early abortion.

**Statistical analysis**

Statistical analysis was performed using SPSS 26.0 software (IBM Inc., United States). Continuous variables were presented as mean ± standard deviation (SD) and Median (Q25, Q75). Categorical variables were presented as count (percentage). Comparisons between groups were determined using nonparametric tests (the Kruskal-Wallis single factor ANOVA test) or chi-squared tests. Multivariate logistic regression analysis was performed to examine the factors such as female age, duration of infertility, cycle number, primary infertility and sperm count after washing that might predict clinical pregnancy and pregnancy loss. All tests were two-sided. The value of \( P < 0.05 \) was used to indicate statistical significance.

**Results**

We studied 1139 IUI cycles from 1139 couples. Patient characteristics were summarized in Table 1. Female age ranged from 22 to 48 years (mean 32.0 ± 3.7 years), and male age ranged from 23 to 60 years (mean 33.5 ± 5.0 years). The mean of sperm DFI (%) and HDS (%) was 15.2 ± 9.2 and 8.6 ± 4.4, respectively. The duration of inability to pregnancy was 2.9 ± 1.9y. The proportion of primary infertility was 57.2% (651/1139), and the proportion of natural cycles was 52.2% (594/1139). Sperm concentration
Sperm concentration after washing was $9.0 \pm 9.8 \times 10^6$/ml. The rate of clinical pregnancy, ectopic pregnancy and pregnancy loss were 9.7% (110/1139), 3.6% (4/110) and 24.5% (27/110) respectively.

Table 1
Data of the studied subjects

| Variable | Cycles, n | 1139 |
|----------|-----------|------|
| DFI, % mean ± SD | 15.2 ± 9.2 |
| Median (Q25, Q75) | 13.4 (8.5, 19.9) |
| HDS, % mean ± SD | 8.6 ± 4.4 |
| Median (Q25, Q75) | 7.8 (5.6, 10.6) |
| Female age, y mean ± SD | 32.0 ± 3.7 |
| Median (Q25, Q75) | 32 (29, 35) |
| Male age, y mean ± SD | 33.5 ± 5.0 |
| Median (Q25, Q75) | 33 (30, 36) |
| Duration of infertility, y mean ± SD | 2.9 ± 1.9 |
| Median (Q25, Q75) | 2(2, 4) |
| Type of infertility, no. (%) | 651(57.2%) |
| Primary infertility | 488(42.8%) |
| Type of treatment cycle, no. (%) | 594(52.2%) |
| natural cycles | 545(47.8%) |
| stimulation cycles | 110/1139 (9.7%) |
| Clinical pregnancy, no./total no. (%) | 4/110 (3.6%) |
| Ectopic pregnancy, no./total no. (%) | 27/110 (24.5%) |
| Pregnancy loss, no./total no. (%) |
The relationship between sperm DNA fragmentation and outcomes of IUI treatment was illustrated in Table 2. Clinical pregnancy rate per cycle in low (DFI < 15%), medium (15% ≤ DFI < 30%), and high (DFI ≥ 30%) sperm DFI groups were 11.1% (74/665), 7.3% (29/395), and 8.9% (7/79), respectively, but no statistical differences were found between the groups (P = 0.127). The pregnancy loss rate in low, medium, and high sperm DFI groups were 24.3% (18/74), 27.6% (8/29), and 14.3% (1/7) with no statistical differences (P = 0.762). However, female age and male age in the group of high sperm DFI were significantly higher than the groups of low and medium sperm DFI (P < 0.001). And the sperm concentration after washing in high sperm DFI group was significantly lower than the groups of low and medium sperm DFI (P < 0.001). No significant differences in the duration of infertility, primary infertility rate, and ectopic pregnancy rate were detected among different groups of sperm DFI levels.
Table 2
Descriptive analysis of different levels of DNA fragmentation index (DFI) for couples receiving IUI treatment.

| Variable                        | DFI                  |   |   | P value |
|---------------------------------|----------------------|---|---|---------|
|                                 | Low (< 15%)          | Medium (≥ 15% and < 30%) | High (≥ 30%) |         |
| Cycle, n                        | 665                  | 395                        | 79           |         |
| Female age, y                   | 31.7 ± 3.8           | 32.4 ± 3.6                 | 33.2 ± 3.8   | < 0.001 |
| Mean ± SD                       | 31 (29, 34) a        | 32 (30, 35) b              | 33 (30, 36) ab |         |
| Median (Q25, Q75)               |                      |                            |              |         |
| Male age, y                     | 32.8 ± 4.6           | 34.1 ± 5.0                 | 36.4 ± 6.4   | < 0.001 |
| Mean ± SD                       | 32 (30, 35)          | 33 (31, 37)                | 35 (32, 39.5) |         |
| Median (Q25, Q75)               |                      |                            |              |         |
| Duration of infertility, y      | 2.9 ± 1.9            | 2.9 ± 2.0                  | 3.0 ± 1.9    | 0.252   |
| Mean ± SD                       | 2 (2, 4)             | 2 (2, 4)                   | 2 (2, 3.5)   |         |
| Median (Q25, Q75)               |                      |                            |              |         |
| Primary infertility, no. (%)    | 384 (57.7%)          | 222 (56.2%)                | 45 (57%)     | 0.886   |
| natural cycles, no. (%)         | 333 (50.1%)          | 226 (57.2%)                | 35 (44.3%)   | 0.028   |
| Sperm concentration after washing, ×10^6/ml | 10.2 ± 10.8 | 7.5 ± 8.3                  | 5.2 ± 4.5    | < 0.001 |
| Mean ± SD                       | 6 (4, 13.5) a        | 5 (2.8, 8.3) b             | 4.2 (2, 6.2) ab |         |
| Median (Q25, Q75)               |                      |                            |              |         |
| Clinical pregnancy, no./total no. (%) | 74/665 (11.1%) | 29/395 (7.3%)              | 7/79 (8.9%)  | 0.127   |
| Ectopic pregnancy, no./total no. (%) | 4/74(5.4%)       | 0                          | 0            | 0.674   |
| Pregnancy loss, no./total no. (%) | 18/74 (24.3%)     | 8/29 (27.6%)               | 1/7 (14.3%)  | 0.872   |

Data are presented as mean ± SD, median (Q25, Q75) or count (percentage). Comparisons between different groups of DFI were determined by Mann–Whitney U test and Chi-squared test, respectively. a. DFI ≥ 30% compared with DFI < 15%. b. DFI ≥ 30% compared with 15% ≤ DFI < 30%. P < 0.05 was considered statistically significant. DFI: DNA fragmentation index.

The relationship between sperm HDS and outcomes of IUI treatment was illustrated in Table 3. Clinical pregnancy rate per cycle in low (HDS ≤ 15%) and high (HDS > 15%) sperm HDS groups were 9.8%
(103/1046) and 7.5% (7/93), with no significant differences between the groups (P = 0.468). The pregnancy loss rates in low and high sperm HDS groups were 26.2% (27/103), 0 (0/7) also with no statistical differences (P = 0.191). Although sperm concentration after washing in the group of high sperm HDS was significantly lower than the group of low sperm HDS (P < 0.001), no significant differences in female age, male age, duration of infertility, primary infertility rate, and ectopic pregnancy rate were detected between the groups of low and high sperm HDS.

Table 3
Descriptive analysis of the different levels of high DNA stainability (HDS) for couples receiving IUI treatment.

| Variable                      | HDS                          |    | P value |
|-------------------------------|------------------------------|----|---------|
|                               | Low (≤ 15%)                  |    |         |
|                               | High (>15%)                  |    |         |
| Cycle, n                      | 1046                         | 93 |         |
| Female age, y                 | 32.1 ± 3.8                   | 31.7 ± 3.5 | 0.242 |
| Mean ± SD                     | 32(29, 35)                   | 31(30, 34) |         |
| Median (Q25, Q75)             |                              |    |         |
| Male age, y                   | 33.5 ± 5.0                   | 33.1 ± 4.6 | 0.724 |
| Mean ± SD                     | 33(30, 36)                   | 33(30, 35) |         |
| Median (Q25, Q75)             |                              |    |         |
| Duration of infertility, y    | 2.9 ± 1.9                    | 2.7 ± 1.6 | 0.454 |
| Mean ± SD                     | 2(2, 4)                      | 2(2, 3)   |         |
| Median (Q25, Q75)             |                              |    |         |
| Primary infertility, no. (%)  | 593 (56.7%)                  | 58 (62.4%) | 0.289 |
| Natural cycles, no. (%)       | 547 (52.3%)                  | 47 (50.5%) | 0.745 |
| Sperm concentration after washing, ×10⁶/ml | 9.2 ± 10.0                  | 5.8 ± 6.8 | < 0.001 |
| Mean ± SD                     | 6(3, 10)                     | 4(2, 7)   |         |
| Median (Q25, Q75)             |                              |    |         |
| Clinical pregnancy, no./total no. (%) | 103/1046 (9.8%)          | 7/93 (7.5%) | 0.468 |
| Ectopic pregnancy, no./total no. (%) | 4/103(3.9%)                 | 0 | 1.00   |
| Pregnancy loss, no./total no. (%) | 27/103 (26.2%)              | 0 | 0.191  |

Data are presented as mean ± SD, median (Q25, Q75) or count (percentage). Comparisons between the two groups of HDS were determined by Mann–Whitney U test and Chi-squared test, respectively. P < 0.05 was considered statistically significant. HDS: high DNA stainability.
Table 4 showed the results of regression models of the association of predicting variables with clinical pregnancy rate and pregnancy loss rate. A statistically significant higher rate of clinical pregnancy was observed with younger age of the female partner (OR = 0.89, 95% CI: 0.82–0.97, P = 0.007) and higher sperm concentration after washing (OR = 1.02, 95% CI: 1.00-1.04, P = 0.042). A higher risk of pregnancy loss was observed with increased female age (OR = 1.43, 95% CI:1.09–1.89, P = 0.010) and lower sperm concentration after washing (OR = 0.92, 95% CI: 0.84–0.99, P = 0.029). The levels of sperm DFI and HDS in semen had no statistically significant effects on clinical pregnancy rate (P = 0.428, P = 0.679, respectively) or pregnancy loss rate (P = 0.761, P = 0.075, respectively).

Table 4
Multivariate logistic regression analyses of factors affecting clinical pregnancy and pregnancy loss.

| Items                                    | Clinical pregnancy | Pregnancy loss |
|------------------------------------------|--------------------|----------------|
|                                          | P value OR (95%CI) | P value OR (95%CI) |
| DFI                                      | 0.428 0.99(0.97, 1.01) | 0.761 1.01(0.95, 1.08) |
| HDS                                      | 0.579 0.97(0.94, 1.04) | 0.075 0.87(0.75, 1.01) |
| Female age                               | 0.007 0.89(0.82, 0.97) | 0.010 1.43(1.09, 1.89) |
| Male age                                 | 0.051 1.06(1.00, 1.12) | 0.107 0.85(0.69, 1.04) |
| Duration of infertility                  | 0.562 0.97(0.86, 1.09) | 0.186 1.21(0.91, 1.60) |
| Secondary infertility (vs. primary infertility) | 0.069 0.68(0.45, 1.03) | 0.986 1.01(0.36, 2.83) |
| Stimulating cycle (vs. nature cycle)     | 0.098 0.71(0.47, 1.07) | 0.650 0.79(0.28, 2.20) |
| Sperm concentration after washing        | 0.042 1.02(1.00, 1.04) | 0.029 0.92(0.84, 0.99) |

Correlations of predicting factors with clinical pregnancy and pregnancy loss were determined by multivariate logistic regression analyses. P< 0.05 was considered statistically significant. DFI: DNA fragmentation index; HDS: high DNA stainability; OR: odds ratio; CI: confidence interval.

Discussion
In this study, we focused on the possible predictive values of SCSA analysis about sperm DNA/chromatin integrity (expressed in terms of DFI and HDS) on the outcomes of IUI. The results indicated that sperm DFI and HDS were not associated with either clinical pregnancy rate or pregnancy loss rate following IUI treatment. However, female age and motile sperm concentration after washing were.
The SCSA analysis is a flow cytometric version of AO staining to determine abnormal sperm chromatin that is highly susceptible to chemically induced in situ partial DNA denaturation[8]. The SCSA test can simultaneously detect single-stranded DNA broken (expressed as DFI) and double-stranded DNA broken (expressed as HDS) (mainly due to impaired replacement of histone with protamines)[9]. The advantage of SCSA is that it has a standardized protocol for all users, minimizing interlaboratory variation, evaluating rapidly a large number of spermatozoa, and assessing many samples. Its disadvantage is that a high cost special equipment, a flow cytometer, is required [10, 11].

Several studies have assessed the association of DFI or HDS measured by SCSA with pregnancy outcomes following IUI. In 2004, Bungum et al. indicated that successful pregnancy and delivery rates were significantly higher in patients with DFI < 27% and HDS < 10%[12]. In 2007, a paper showed the ORs for biochemical pregnancy, clinical pregnancy and delivery were significantly lower for couples with DFI > 30% [13]. Then in 2008, it was found that those who achieved clinical pregnancy and those who did not had no significantly different levels of sperm DFI and HDS [14]. In 2011, it was suggested sperm DNA fragmentation was a robust indicator for IUI outcomes with a cut-off DFI of 25%[15]. Nevertheless, the sample sizes in these studies were small, the results were controversial, and their proposed cut-offs of DFI and HDS were different. A meta-analysis by Sugihara used these four studies above and analyzed the association between sperm DNA fragmentation and IUI pregnancy in relative risk. The author suggested that the current literature could not offer enough evidence to determine the use of SCSA as a routine medical-diagnostic test for couples undergoing IUI[16].

Our study obtained 1139 IUI cycles (only one treatment cycle per couple was included). The clinical pregnancy rates per cycle and the pregnancy loss rates among high, medium, and low sperm DFI groups did not have statistical differences (P = 0.127, P = 0.762). In addition, the clinical pregnancy rate per cycle and the pregnancy loss rate in low (HDS ≤ 15%) and high (HDS > 15%) did not have significant differences (P = 0.468, 0.191). In our data, the main factors significantly affecting IUI outcomes were female age and sperm concentration.

Whether sperm DFI can predict the IUI outcomes and whether can the detection and improvement of sperm DFI could contribute to a successful pregnancy of ART are controversial. Various reasons can be considered contributing to the controversy. Firstly, sperm quality is subject to multiple factors with great fluctuations. In fact, sperm DNA damage may be linked to both intrinsic causes (deficiencies in recombination during spermatogenesis, abnormal spermatid maturation, protamine 1 and 2 ratios, abortive apoptosis, and oxidative stress) and extrinsic causes (age, obesity, alcohol, smoking, abstinence time, handling procedure, storage temperature and cryopreservation, post-testicular oxidative stress, varicocele, infections, drugs, occupational exposure to toxic agents, episodic air pollution, and so on)[17, 10, 18]. Therefore, the exact cause of sperm DNA damage could not be determined. Secondly, the controversy could be imputable to the heterogeneous nature of the studies conducted using various assays to evaluate sperm DNA fragmentation (SDF). The most commonly used tests were the sperm chromatin structure assay (SCSA), terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL), sperm chromatin dispersion (SCD), and single cell gel electrophoresis assay (Comet)[19]. To
some extent, the results from different SDF assays were generally not comparable due to the different aspects of SDF measured[20]. Moreover, the lack of a standardized protocol for specimen collection and a clear threshold value for SDF assays is troubling[19]. Thirdly, additional factors affect the measured outcomes of ART, including female age and fertility status[11].

Our data showed that while DFI value was not associated with IUI outcomes, female age was. We speculated that DNA repair was a possible reason behind the observation. Sperm DNA strand breaks can be repaired during spermiogenesis[21, 22], in the fertilized oocyte[23, 24] and during early embryonic development[25, 26]. If DNA damage failed in gametogenic DNA repair or if DNA damage occurs in the spermatozoa owing to extrinsic factors, the damage can be successfully repaired during fertilization[27, 28]. The capacity of DNA repair in the early fertilized embryo relies entirely on the maternal mRNAs and proteins deposited and stored in the oocyte before ovulation. If the oocyte is not adequately equipped, or if the zygotic gene expression does not start at the correct time, the repair to sperm DNA may fail and the embryo will die [29]. Thus, a successful pregnancy probably depends to a great extent on the quality of oocytes, partly because the amount of damage correction is higher in good quality oocytes. And female age is the most important factor affecting oocyte quality[30, 31]. As maternal age increases, the mRNA stored in oocytes and the efficiency of DNA repair decrease[32]. Therefore, our result is consistent showing that women's age is negatively correlated with IUI pregnancy outcomes. In addition to female age, our data showed that motile sperm concentration after washing significantly affected IUI outcomes. It has been reported by many previous studies that the number of motile sperm inseminated could be used as a predictor of success in IUI[33, 34]. There was a trend toward an increasing percent of conception with increasing total motile sperm count[35]. Sperm DFI was negatively related to sperm concentration, sperm motility, and total normal-progressively motile sperm count[36, 37]. Therefore, DFI is a potential confounding variable in this case. The negative relation between clinical pregnancy rates and sperm DFI found in previous studies may partly be due to the less motile sperm count.

**Conclusion**

Sperm DFI and HDS were not significantly correlated with clinical pregnancy and pregnancy loss in cycles of IUI. Female age and motile sperm count, which were significantly different between groups of different levels of DFI and HDS, had statistically significant effects on clinical pregnancy and pregnancy loss after IUI treatment. We suggested that the evaluation of sperm DFI and HDS by SCSA was not essential to IUI performance.

**Declarations**

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Availability of data and materials

All data that were generated or analyzed during this study are included in this publication. Detailed clinical information of the patients reported in this paper are available from the corresponding author on reasonable request.

Author contributions

JMG was the major contributor in writing the manuscript, collecting and analyzing the data. JQ, LYY contributed to conceive and design the experiments; YH and YYW helped in summarizing and analyzing the data. HJ, LYY and JQ performed a critical review and edition of the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This was a retrospective study and was approved by the Institutional Review Board of Peking University Third Hospital, China.

Consent for publication

All authors commented on and approved the manuscript.

Competing interests

The authors declare that they have no competing interests.

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