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CIRCULATING GALECTIN-3, HMGB1, SURVIVIN, VEGF, GDF-15 AND PENTRAXIN-3 AS DISCRIMINATORY BIOMARKERS ACROSS HEALTHY CONTROLS, INFERTILE MEN AND PATIENTS WITH TESTICULAR CANCER: A COMPARATIVE CROSS-SECTIONAL STUDY FROM NAJAF, IRAQ

[1]Nisreen Qasim Shakir

[1]Department of Pharmacognosy and Medicinal Plants, College of Pharmacy, University of Kufa, Iraq

Corresponding Author:
nisreenq.alfatn@uokufa.edu.iq

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ABSTRACT

Background: *Male infertility and testicular cancer share developmental and inflammatory roots, yet no single panel of circulating proteins has been evaluated simultaneously across both conditions. We measured six serum biomarkers spanning inflammation, apoptosis inhibition and angiogenesis in three clinically distinct groups.*

Methods: *In this cross-sectional study of 180 men recruited in Najaf, Iraq (60 healthy fertile controls, 60 infertile men and 60 patients with testicular cancer), serum galectin-3, high mobility group box 1 (HMGB1), survivin, vascular endothelial growth factor (VEGF), growth differentiation factor-15 (GDF-15) and pentraxin-3 were quantified by enzyme-linked immunosorbent assay. Groups were compared with Welch analysis of variance and Games-Howell post-hoc testing; discrimination was assessed by receiver-operating-characteristic analysis and logistic regression.*

Results: *All six biomarkers rose progressively from controls, through infertile men, to patients with testicular cancer (all $P < 0.001$), with large effect sizes. Concentrations correlated inversely with sperm concentration, motility and morphology and directly with follicle-stimulating hormone. For separating testicular cancer from controls, areas under the curve ranged from 0.879 to 0.951, and a cross-validated six-marker panel reached 0.989; the panel reached 0.948 for infertile men versus controls and 0.863 for testicular cancer versus infertile men.*

Conclusions: *In this single-centre sample, the six circulating proteins were graded across the reproductive-health spectrum and discriminated the study groups with good accuracy. The findings are hypothesis-generating and require external, prospective validation before any clinical application.*

KEYWORDS: *Male Infertility; Testicular Cancer; Galectin-3; Hmgb1; Survivin; Vegf; Gdf-15; Pentraxin-3; Iraq*

INTRODUCTION

Infertility affects roughly one in seven couples worldwide, and a male factor contributes to about half of these cases.[1] Beyond its immediate reproductive consequences, impaired spermatogenesis is increasingly recognised as a marker of broader male health, including a measurable excess risk of certain malignancies.[1] The most consistent of these associations is with testicular cancer. Testicular germ-cell tumours are the most common solid malignancy of young men, with an age-standardised incidence of about 1.7 per 100,000 worldwide in 2022, and although they are highly curable they impose a substantial burden because they strike during the reproductive years.[2]

The link between subfertility and testicular cancer is not merely statistical. The testicular dysgenesis syndrome hypothesis proposes that cryptorchidism, hypospadias, poor semen quality and germ-cell neoplasia share a common origin in disturbed fetal testicular development.[3] Consistent with this idea, population-based cohorts and meta-analyses report that infertile men carry roughly twice the risk of subsequently developing testicular cancer compared with fertile men.[4,5,6] Both conditions are also characterised by a disturbed testicular micro-environment in which chronic inflammation, dysregulated apoptosis and abnormal angiogenesis feature prominently, and several developmental proteins detectable in serum and seminal plasma have been proposed as shared read-outs of this disturbance.[7]

Galectin-3 is a β-galactoside-binding lectin that participates in cell proliferation, apoptosis, inflammation and fibrosis, is released into serum during tissue injury, and has been advanced as an early-stage biomarker across several diseases.[8] In men, serum and seminal galectin-3 are elevated in azoospermia and correlate inversely with testosterone, implicating it in defective spermatogenesis.[9] High mobility group box 1 (HMGB1) is a prototypical damage-associated molecular pattern; once released from stressed or dying cells it promotes testicular inflammation and germ-cell apoptosis through the Bcl-2/Bax axis, and its neutralisation ameliorates experimental orchitis.[10,11,12] Survivin, a member of the inhibitor-of-apoptosis family, is expressed in a stage-specific manner during spermatogenesis and is strongly re-expressed in testicular germ-cell tumours, where it suppresses apoptosis and supports tumour-cell survival.[13] Vascular endothelial growth factor (VEGF) governs testicular angiogenesis and is up-regulated in varicocele and other infertile states, while also driving tumour neovascularisation.[18] Growth differentiation factor-15 (GDF-15), a stress-responsive member of the transforming growth factor-β superfamily, rises with oxidative stress, inflammation and endothelial damage and behaves as a biomarker and immune checkpoint across malignancies, including testicular cancer treated with platinum-based chemotherapy.[14,15] Finally, pentraxin-3 (PTX3) is a long pentraxin that links innate immunity, inflammation and reproduction and whose elevated expression is associated with poorer survival across human cancers.[16,17]

Despite this mechanistic plausibility, the evidence base remains fragmented. Individual markers have usually been studied in a single condition and against a single comparison group; the available data are dominated by tissue or seminal-plasma measurements rather than readily accessible serum; and very few studies have examined the same panel of proteins simultaneously in infertile men and in men with testicular cancer. To our knowledge, no previous study has measured galectin-3, HMGB1, survivin, VEGF, GDF-15 and PTX3 together across healthy fertile controls, infertile men and patients with testicular cancer, and none has done so in an Iraqi population, where the burden of male infertility is appreciable but circulating-biomarker data are scarce.[24,25,26]

We therefore conducted a cross-sectional study to characterise the serum concentrations of these six biomarkers across the three groups, to test whether they vary in a graded fashion along this reproductive-health spectrum, to examine their relationships with conventional semen and hormonal parameters, and to explore their ability to discriminate between groups. The work was framed as hypothesis-generating: the aim was to map the behaviour of the panel rather than to propose a ready-to-use diagnostic test.

MATERIALS AND METHODS

STUDY SUBJECT

This single-centre, cross-sectional comparative study was carried out in Najaf Al-Ashraf, Iraq, between November 2022 and February 2024. Participants were recruited from the urology and oncology departments and the outpatient fertility services of Al-Sadr Teaching Hospital, Al-Hakeem General Hospital and Al-Najaf Teaching Hospital, together with collaborating private andrology and urology clinics in Najaf.

PARTICIPANTS AND GROUPING

A total of 180 men were enrolled and allocated to three equally sized groups of 60. The healthy control group comprised fertile men of proven paternity or with normal semen parameters and no history of reproductive disorders. The infertile group comprised men with primary or secondary infertility, defined as failure to achieve a pregnancy after at least twelve months of regular unprotected intercourse, in whom a female factor had been excluded or addressed. The testicular cancer group comprised men with a histologically confirmed testicular germ-cell tumour; in participants sampled after orchidectomy, blood was drawn before the start of any systemic chemotherapy or radiotherapy in order to avoid treatment-related confounding of biomarker levels.

Men were excluded if they had another active malignancy, a current acute infection or febrile illness, a known chronic inflammatory or autoimmune disease, decompensated hepatic or renal disease, or if they had received cytotoxic chemotherapy, radiotherapy or systemic immunomodulatory drugs before sampling. These criteria were applied to limit the influence of conditions known to alter the inflammatory, apoptotic and angiogenic markers under study.

Demographic, anthropometric and clinical data were collected by structured interview and review of medical records, including age, residence, marital status, education, occupation, income, smoking status, alcohol use, physical activity (graded with the International Physical Activity Questionnaire), family history of infertility and cancer, and relevant andrological history. Erectile function was assessed with the five-item International Index of Erectile Function. Body mass index was calculated as weight in kilograms divided by height in metres squared, using a stadiometer and a calibrated digital scale; waist circumference was measured at the umbilicus with a non-elastic tape. Blood pressure and heart rate were recorded with a validated automated digital sphygmomanometer after a rest period.

TUMOUR CLASSIFICATION AND STAGING

For the testicular cancer group, tumour characteristics were retrieved from histopathology and imaging records. Histological type was classified as seminoma, non-seminoma or mixed germ-cell tumour according to the current World Health Organization classification of urinary and male genital tumours.[21] Clinical stage was assigned with the American Joint Committee on Cancer tumour-node-metastasis-serum-marker system on the basis of physical examination, scrotal ultrasonography, computed tomography and serum tumour markers.[22] Tumour diameter, affected side and the presence of metastasis were recorded.

SEMEN ANALYSIS

Semen samples were produced by masturbation after two to five days of sexual abstinence and were examined after liquefaction in accordance with the sixth edition of the World Health Organization laboratory manual for the examination and processing of human semen.[19,20] Macroscopic assessment included volume, measured with a graduated collection tube, and pH, measured with a calibrated pH meter. Sperm concentration and total count were determined with an improved Neubauer haemocytometer, and motility was graded by light microscopy as progressive, non-progressive or immotile. Sperm morphology was evaluated on Papanicolaou-stained smears applying strict (Kruger) criteria, vitality was assessed by eosin-nigrosin staining, and seminal leukocytes were quantified by peroxidase staining. The lower reference limits of the sixth-edition manual were used as the interpretive framework (sperm concentration 16 million per millilitre, total count 39 million per ejaculate, progressive motility 30 per cent, total motility 42 per cent, normal morphology 4 per cent and vitality 54 per cent).

BLOOD SAMPLING, HAEMATOLOGY AND BIOCHEMISTRY

Venous blood was collected between 8 and 11 a.m. after an overnight fast. Samples for serum biomarker and hormone assays were drawn into plain gel tubes, allowed to clot, and centrifuged at 3000 revolutions per minute for ten minutes; the separated serum was aliquoted and stored at −80 °C until analysis, and repeated freeze-thaw cycles were avoided. A complete blood count was performed on EDTA-anticoagulated blood with an automated haematology analyser (Mindray BC-5150; Shenzhen Mindray Bio-Medical Electronics, Shenzhen, China). Fasting glucose, urea, creatinine, alanine and aspartate aminotransferases, albumin, total protein and C-reactive protein were measured on an automated clinical chemistry analyser (Cobas c311; Roche Diagnostics, Mannheim, Germany) using the manufacturer's enzymatic, kinetic and immunoturbidimetric methods, with creatinine measured by the Jaffe reaction.

REPRODUCTIVE HORMONE ASSAYS

Reproductive hormones were quantified in serum by enzyme-linked immunosorbent assay, read on a microplate reader (Human Reader HS; HUMAN Diagnostics, Wiesbaden, Germany) with an automated plate washer, following each manufacturer's protocol. Total testosterone (BioVendor, Brno, Czech Republic; catalogue RCD027R) and free testosterone (ALPCO, Salem, NH, USA; catalogue 11-FTEHU-E01) were measured by competitive ELISA. Follicle-stimulating hormone (Elabscience, Wuhan, China; catalogue E-EL-H1143) and luteinising hormone (Elabscience; catalogue E-EL-H6019) were measured by sandwich ELISA. Prolactin (R&D Systems, Minneapolis, MN, USA; catalogue DPRL00), estradiol (R&D Systems; catalogue KGE014), inhibin B (Ansh Labs, Webster, TX, USA; catalogue AL-107) and anti-Mullerian hormone (Ansh Labs; catalogue AL-105) were measured with their respective kits. All samples were assayed in duplicate and reported as the mean.

MEASUREMENT OF THE SIX CANDIDATE BIOMARKERS

The six candidate biomarkers were measured in serum by commercially available sandwich ELISA kits according to the manufacturers' instructions, with standards and samples assayed in duplicate on the same plate reader. Galectin-3 (Elabscience, Wuhan, China; catalogue E-EL-H1470), survivin (Elabscience; catalogue E-EL-H1584) and pentraxin-3 (Elabscience; catalogue E-EL-H6081) were measured with their respective kits; HMGB1 was measured with the IBL International kit (Hamburg, Germany; catalogue ST51011); and VEGF (catalogue DVE00) and GDF-15 (catalogue DGD150) were measured with Quantikine ELISA kits (R&D Systems, Minneapolis, MN, USA). Optical density was read at the recommended wavelength, concentrations were interpolated from a standard curve constructed for each plate, and values were expressed in nanograms per millilitre for galectin-3 and HMGB1 and in picograms per millilitre for survivin, VEGF, GDF-15 and pentraxin-3. Assays were performed by an operator blinded to group allocation.

STATISTICAL ANALYSIS

Data were analysed with standard statistical software, and two-sided P values below 0.05 were considered statistically significant. The distribution of continuous variables was assessed with the Shapiro-Wilk test and variance homogeneity with the Levene test. Normally distributed variables are summarised as mean ± standard deviation and were compared

across groups by one-way analysis of variance, with the Welch correction applied when variances were unequal; non-normally distributed variables are summarised as median with interquartile range and were compared by the Kruskal-Wallis test. Because the six biomarkers were approximately normally distributed within groups but showed unequal variances, they were compared by Welch analysis of variance with Games-Howell post-hoc testing, and the η^2 statistic was used to express effect size. Categorical variables are reported as counts and percentages and were compared by the chi-square or Fisher exact test.

Associations between biomarkers and semen and hormonal parameters were examined with the Spearman rank correlation coefficient across the whole cohort. Diagnostic performance was evaluated by receiver-operating-characteristic analysis for each pairwise contrast; the area under the curve was reported with a bootstrap 95 per cent confidence interval (2000 resamples), and optimal cut-offs were chosen by maximising the Youden index.[23] A combined six-marker score was generated by multivariable logistic regression with stratified five-fold cross-validation to limit optimistic bias. The independent association of each biomarker with disease status was summarised by univariable logistic regression, expressed as the odds ratio per one-standard-deviation increase in biomarker concentration.

RESULTS

The three groups were comparable in most baseline characteristics (Table 1). Mean age did not differ significantly (controls 31.9 ± 4.8 years, infertile 33.4 ± 5.7 years, testicular cancer 31.6 ± 6.0 years; P = 0.173), and there were no significant differences in blood pressure, heart rate or residence. Smoking status was balanced by design (40 per cent smokers in each group). Body mass index differed modestly (P = 0.049) and waist circumference showed a borderline difference (P = 0.053), with slightly higher values among infertile men. A family history of infertility was more frequent in the infertile group (21.7 per cent) than in controls (5.0 per cent) or testicular cancer patients (6.7 per cent; P = 0.006), whereas a family history of cancer was uncommon and similar across groups.

Table 1: Baseline demographic and clinical characteristics of the study population across the three groups.

Characteristic	Control (n = 60)	Infertile (n = 60)	Testicular cancer (n = 60)	P value	Test
Age (years)	31.9 ± 4.8	33.4 ± 5.7	31.6 ± 6.0	0.173	ANOVA
Body mass index (kg/m²)	26.0 [22.6–30.2]	27.8 [25.5–30.8]	26.5 [21.9–29.1]	0.049	Kruskal–Wallis
Systolic blood pressure (mmHg)	117.5 [110.8–122.2]	117.0 [108.0–125.0]	120.5 [109.0–128.2]	0.383	Kruskal–Wallis
Diastolic blood pressure (mmHg)	76.0 ± 9.4	75.9 ± 8.4	75.7 ± 9.0	0.979	ANOVA
Heart rate (bpm)	71.2 ± 10.3	74.5 ± 9.0	71.4 ± 8.3	0.098	ANOVA
Waist circumference (cm)	89.3 [81.0–97.0]	93.5 [86.7–100.1]	90.0 [82.4–95.6]	0.053	Kruskal–Wallis
BMI category				**0.142**	**Chi-square**
Normal	23 (38.3)	14 (23.3)	25 (41.7)		
Overweight	21 (35.0)	27 (45.0)	25 (41.7)		
Obese	16 (26.7)	19 (31.7)	10 (16.7)		
Residence				**0.436**	**Chi-square**
Urban	41 (68.3)	39 (65.0)	40 (66.7)		
Town	12 (20.0)	8 (13.3)	7 (11.7)		
Rural	7 (11.7)	13 (21.7)	13 (21.7)		
Smoking status				**1.000**	**Chi-square**
Non-smoker	36 (60.0)	36 (60.0)	36 (60.0)		
Smoker	24 (40.0)	24 (40.0)	24 (40.0)		
Alcohol consumption				**0.167**	**Fisher's exact**
Never	59 (98.3)	57 (95.0)	60 (100.0)		
Occasional	1 (1.7)	3 (5.0)	0 (0.0)		
Physical activity				**0.858**	**Chi-square**
High	7 (11.7)	4 (6.7)	6 (10.0)		
Moderate	18 (30.0)	16 (26.7)	16 (26.7)		
Low	35 (58.3)	40 (66.7)	38 (63.3)		
Family history of infertility				**0.006**	**Chi-square**
No	57 (95.0)	47 (78.3)	56 (93.3)		
Yes	3 (5.0)	13 (21.7)	4 (6.7)		
Family history of cancer				**0.862**	**Fisher's exact**
No	58 (96.7)	58 (96.7)	57 (95.0)		
Yes	2 (3.3)	2 (3.3)	3 (5.0)		

Data are presented as mean ± standard deviation for normally distributed continuous variables, median [interquartile range] for non-normally distributed variables, and n (%) for categorical variables.

P values were derived from one-way ANOVA for continuous variables and from the chi-square or Fisher exact test for categorical variables, as indicated in the Test column. BMI, body mass index; bpm, beats per minute. A two-sided P < 0.05 was considered statistically significant.

The reproductive-hormone profile distinguished the groups in a pattern typical of each condition (Table 2). Infertile men showed the expected features of impaired spermatogenesis, with the lowest total and free testosterone, the highest follicle-stimulating and luteinising hormones, and a markedly elevated prolactin (24.38 ± 10.48 ng/mL, compared with about 10 ng/mL in the other groups; P < 0.001). Inhibin B fell progressively from controls (162.1 ± 71.1 pg/mL) through infertile men (108.4 ± 57.8 pg/mL) to testicular cancer patients (89.5 ± 45.4 pg/mL; P < 0.001), and anti-Mullerian hormone was likewise highest in controls (P = 0.001). As expected, every semen parameter was most severely affected in the infertile group and intermediate in the testicular cancer group: sperm concentration fell from a median of 53.4 million per millilitre in controls to 8.6 in infertile men and 14.6 in testicular cancer patients, and progressive motility, morphology and vitality followed the same order (all P < 0.001).

Table 2: Haematological, biochemical, hormonal and semen-analysis parameters across the three groups.

Parameter	Control (n = 60)	Infertile (n = 60)	Testicular cancer (n = 60)	P value	Test
Haematology and biochemistry					
Haemoglobin (g/dL)	15.2 ± 0.9	15.1 ± 0.9	14.9 ± 1.1	0.181	ANOVA
White blood cells (×10³/µL)	6.7 ± 1.3	6.7 ± 1.3	6.1 ± 1.3	0.018	ANOVA
Platelets (×10³/µL)	244.1 ± 48.4	242.6 ± 49.1	246.3 ± 49.9	0.917	ANOVA
C-reactive protein (mg/L)	1.53 ± 0.77	3.95 ± 1.78	8.75 ± 4.19	<0.001	Welch ANOVA
Fasting glucose (mg/dL)	88.7 [80.8–97.4]	90.9 [86.5–97.7]	87.5 [83.2–94.8]	0.084	Kruskal–Wallis
Creatinine (mg/dL)	0.91 ± 0.13	0.92 ± 0.15	0.91 ± 0.17	0.865	ANOVA
ALT (U/L)	28.6 ± 9.2	29.3 ± 9.0	29.0 ± 9.9	0.933	ANOVA
AST (U/L)	24.6 [19.6–30.7]	22.4 [18.9–29.1]	22.3 [16.9–26.9]	0.171	Kruskal–Wallis
Reproductive hormones					
Testosterone (ng/mL)	4.46 ± 1.42	2.75 ± 1.00	4.06 ± 1.69	<0.001	Welch ANOVA
Free testosterone (pg/mL)	125.2 [73.5–151.7]	71.2 [54.4–92.8]	121.6 [83.2–152.3]	<0.001	Kruskal–Wallis
FSH (IU/L)	3.86 ± 1.63	10.62 ± 5.54	7.67 ± 4.02	<0.001	Welch ANOVA
LH (IU/L)	4.21 [3.42–5.21]	6.25 [4.72–7.95]	5.29 [3.48–6.94]	<0.001	Kruskal–Wallis
Prolactin (ng/mL)	9.78 ± 2.94	24.38 ± 10.48	10.78 ± 3.54	<0.001	Welch ANOVA
Estradiol (pg/mL)	26.6 ± 7.1	31.5 ± 10.4	29.6 ± 7.8	0.007	Welch ANOVA
Inhibin B (pg/mL)	162.1 ± 71.1	108.4 ± 57.8	89.5 ± 45.4	<0.001	Welch ANOVA
AMH (ng/mL)	7.17 ± 3.52	5.31 ± 2.70	5.41 ± 2.87	0.001	ANOVA
Semen analysis					
Semen volume (mL)	3.15 ± 0.81	2.52 ± 1.13	2.85 ± 1.02	0.003	Welch ANOVA
Semen pH	7.52 ± 0.28	7.35 ± 0.35	7.41 ± 0.30	0.013	ANOVA
Sperm concentration (×10⁶/mL)	53.4 [37.3–62.8]	8.6 [1.1–11.9]	14.6 [3.3–28.7]	<0.001	Kruskal–Wallis
Total sperm count (×10⁶)	154.3 [97.4–233.2]	14.1 [2.0–33.9]	41.2 [4.6–90.9]	<0.001	Kruskal–Wallis
Progressive motility (%)	51.7 [40.2–58.5]	15.1 [8.7–24.6]	33.1 [22.1–46.4]	<0.001	Kruskal–Wallis
Total motility (%)	64.0 ± 8.5	35.3 ± 10.9	48.5 ± 12.6	<0.001	Welch ANOVA
Normal morphology (%)	16.5 ± 6.7	3.0 ± 1.5	6.9 ± 3.5	<0.001	Welch ANOVA
Vitality (%)	71.3 [63.9–75.7]	43.5 [33.7–53.6]	57.6 [49.3–67.1]	<0.001	Kruskal–Wallis

Data are presented as mean ± standard deviation for normally distributed variables and as median [interquartile range] for non-normally distributed variables; the corresponding omnibus test is given in the Test column.

ALT, alanine aminotransferase; AMH, anti-Mullerian hormone; AST, aspartate aminotransferase; FSH, follicle-stimulating hormone; LH, luteinising hormone. A two-sided P < 0.05 was considered statistically significant.

The six candidate biomarkers were the central finding of the study (Table 3, Figure 1). All increased in a stepwise manner from controls, through infertile men, to patients with testicular cancer, and every omnibus comparison was highly significant (all $P < 0.001$). Mean galectin-3 concentrations were 8.26 ± 2.43, 13.67 ± 4.19 and 17.74 ± 5.88 ng/mL across the three groups; HMGB1 rose from 2.47 to 5.05 to 6.87 ng/mL; survivin from 49.4 to 89.3 to 142.7 pg/mL; VEGF from 224 to 343 to 502 pg/mL; GDF-15 from 473 to 654 to 895 pg/mL; and pentraxin-3 from 1.69 to 3.28 to 5.07 pg/mL. Effect sizes were large, with η^2 values ranging from 0.334 for GDF-15 to 0.464 for survivin.

Tumour characteristics of the testicular cancer group are summarised in Table 4. Seminoma was the most common histology (50.0 per cent), followed by non-seminomatous (40.0 per cent) and mixed germ-cell tumours (10.0 per cent). Most patients presented with localised disease (stage I, 58.3 per cent), the right testis was slightly more often affected (53.3 per cent), and distant metastasis was present in 28.3 per cent. The mean maximum tumour diameter was 33.4 ± 15.2 mm, and a family history of testicular cancer was rare (1.7 per cent).

Table 3: Serum concentrations of the six candidate biomarkers across the three study groups, with omnibus testing, effect size and pairwise post-hoc analysis.

Biomarker	Control	Infertile	Testicular cancer	F	P value	η^2	C vs I	C vs TC	I vs TC
Galectin-3 (ng/mL)	8.26 ± 2.43	13.67 ± 4.19	17.74 ± 5.88	87.6	<0.001	0.442	<0.001	<0.001	<0.001
HMGB1 (ng/mL)	2.47 ± 1.02	5.05 ± 2.04	6.87 ± 2.85	88.2	<0.001	0.427	<0.001	<0.001	<0.001
Survivin (pg/mL)	49.39 ± 20.23	89.30 ± 39.64	142.71 ± 56.40	85.5	<0.001	0.464	<0.001	<0.001	<0.001
VEGF (pg/mL)	224.2 ± 88.3	343.4 ± 133.4	501.7 ± 144.4	82.7	<0.001	0.459	<0.001	<0.001	<0.001
GDF-15 (pg/mL)	472.7 ± 144.6	654.1 ± 218.5	894.9 ± 336.1	45.8	<0.001	0.334	<0.001	<0.001	<0.001
Pentraxin-3 (pg/mL)	1.69 ± 0.73	3.28 ± 1.63	5.07 ± 2.63	62.5	<0.001	0.364	<0.001	<0.001	<0.001

Data are presented as mean ± standard deviation (ng/mL for galectin-3 and HMGB1; pg/mL for survivin, VEGF, GDF-15 and pentraxin-3). The three right-hand columns give post-hoc P values.

Because all biomarkers were approximately normally distributed within groups (Shapiro-Wilk $P > 0.05$) but showed unequal variances (Levene $P < 0.05$), the omnibus comparison used Welch ANOVA and pairwise post-hoc comparisons used the Games-Howell test.

F, Welch F statistic; η^2, η^2 effect size (0.01 small, 0.06 medium, 0.14 large); C, control; I, infertile; TC, testicular cancer. A two-sided $P < 0.05$ was considered statistically significant.

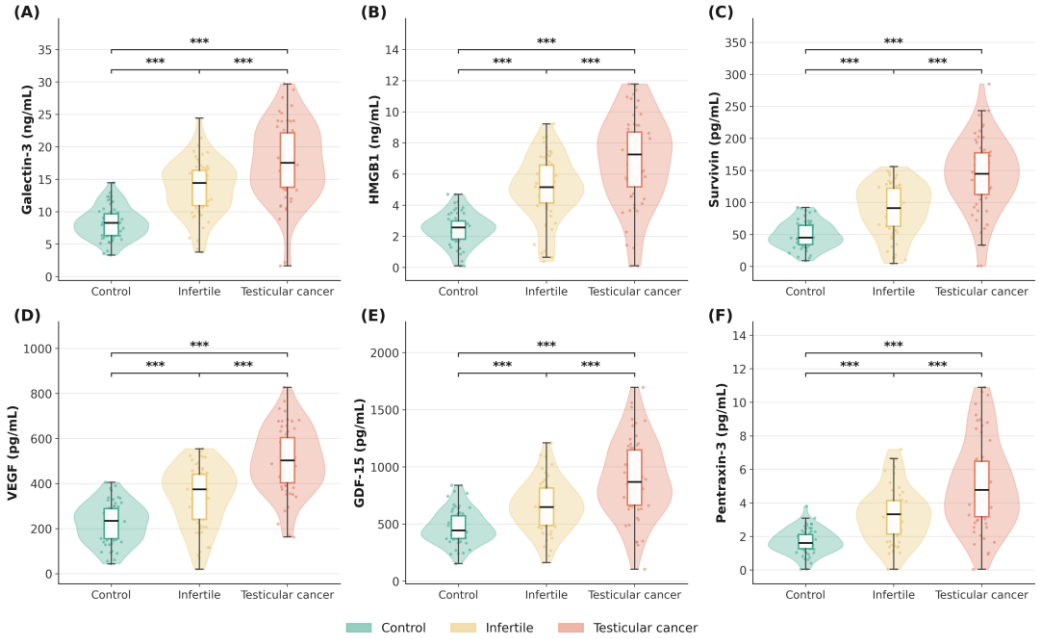

Figure 1: Distribution of the six candidate biomarkers across the three study groups. Violin plots show the full distribution; overlaid box plots indicate the median and interquartile range, with individual data points jittered for transparency. Brackets denote pairwise comparisons (Games-Howell post-hoc test): ***P < 0.001. Groups: green, control; amber, infertile; coral, testicular cancer. (A) Galectin-3; (B) HMGB1; (C) Survivin; (D) VEGF; (E) GDF-15; (F) Pentraxin-3.

Table 4: Tumour characteristics of the testicular cancer group

Characteristic	Category	n (%)
Histological type	Seminoma	30 (50.0)
	Non-seminoma	24 (40.0)
	Mixed	6 (10.0)
Tumour stage	Stage I	35 (58.3)
	Stage II	8 (13.3)
	Stage III	17 (28.3)
Affected side	Right	32 (53.3)
	Left	26 (43.3)
	Bilateral	2 (3.3)
Metastasis	No	43 (71.7)
	Yes	17 (28.3)
Family history of testicular cancer	No	59 (98.3)
	Yes	1 (1.7)
Tumour size (mm)	mean ± SD	33.4 ± 15.2

Data are presented as n (%) for categorical variables and mean ± standard deviation for tumour size. Percentages are calculated relative to the testicular cancer group (n = 60). SD, standard deviation.

Across the whole cohort, the six biomarkers were consistently related to conventional markers of testicular function (Table 5, Figure 3). Galectin-3 showed the strongest associations, correlating with sperm concentration (ρ = −0.62) and with follicle-stimulating hormone (ρ = +0.62), both highly significant. HMGB1 behaved similarly (sperm concentration ρ = −0.57; follicle-stimulating hormone ρ = +0.53). VEGF, GDF-15 and pentraxin-3 showed weaker but still significant correlations, and their associations with testosterone were small and inconsistent.

Table 5: Spearman rank correlations between the six biomarkers and key semen and reproductive-hormone parameters across the whole cohort.

Biomarker	Sperm conc.	Total count	Prog. motility	Normal morph.	Vitality	Testos-terone	FSH	Inhibin B
Galectin-3	−0.62***	−0.59***	−0.51***	−0.39***	−0.24**	−0.36***	0.62***	−0.48***
HMGB1	−0.57***	−0.53***	−0.38***	−0.41***	−0.30***	−0.25***	0.53***	−0.44***
Survivin	−0.51***	−0.44***	−0.35***	−0.31***	−0.22**	−0.22**	0.35***	−0.37***
VEGF	−0.50***	−0.44***	−0.35***	−0.29***	−0.24**	−0.10	0.35***	−0.40***
GDF-15	−0.43***	−0.39***	−0.31***	−0.27***	−0.20**	−0.14	0.32***	−0.33***
Pentraxin-3	−0.38***	−0.34***	−0.24**	−0.36***	−0.34***	−0.04	0.23**	−0.20**

*Values are Spearman rank correlation coefficients (ρ), computed across all 180 participants. Significance: *P < 0.05; **P < 0.01; ***P < 0.001.*

Negative coefficients indicate an inverse association (higher biomarker concentrations with poorer semen quality); positive coefficients with FSH indicate a direct association. FSH, follicle-stimulating hormone.

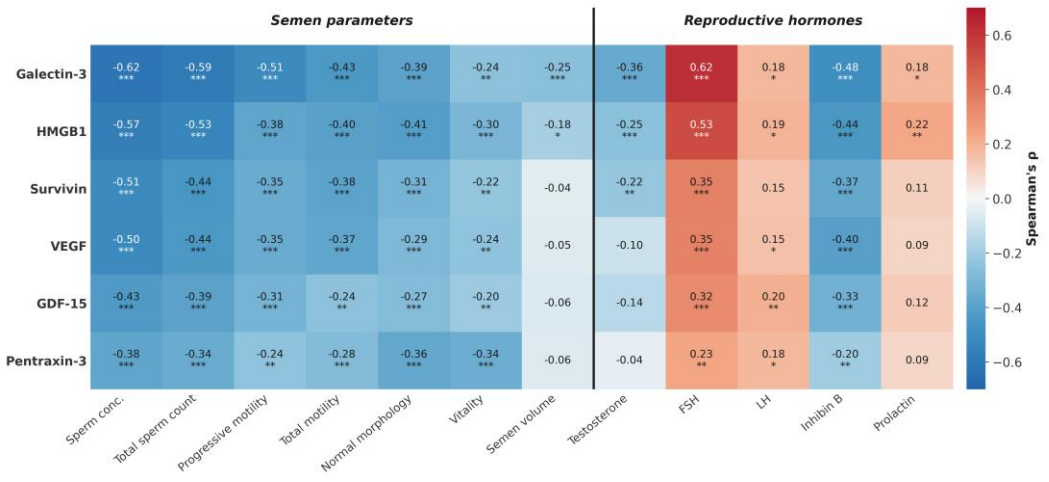

Figure 3: Correlation heatmap between biomarkers and semen and reproductive-hormone parameters. Cells show the Spearman rank correlation coefficient (ρ) computed across all 180 participants; colour intensity reflects the strength and direction of the association (blue, negative; red, positive). Asterisks denote statistical significance: *P < 0.05; **P < 0.01; ***P < 0.001. The vertical divider separates semen parameters (left) from reproductive hormones (right).

The ability of the biomarkers to separate the groups was assessed by receiver-operating-characteristic analysis (Table 6, Figure 2). For distinguishing testicular cancer from controls, individual areas under the curve were high, ranging from 0.879 for GDF-15 to 0.951 for VEGF, with galectin-3 (0.941) and survivin (0.933) also performing strongly. Combining the six markers in a cross-validated logistic model raised the area under the curve to 0.989, with a sensitivity of 91.7 per cent and a specificity of 100 per cent at the selected threshold. Discrimination of infertile men from controls was good but more modest, with individual areas under the curve between 0.761 and 0.870 and a combined-panel value of 0.948. As anticipated, separating testicular cancer from infertile men was the most difficult task, since both groups share an inflamed and dysfunctional testicular environment; individual areas under the curve fell to between 0.702 and 0.789, and the combined panel achieved 0.863.

The univariable logistic-regression analysis reinforced these findings (Table 7, Figure 4). Expressed per one-standard-deviation increase, every biomarker was strongly associated with testicular cancer relative to controls, with odds ratios ranging from about 12 for GDF-15 to about 54 for pentraxin-3; the wide confidence intervals reflect the near-complete separation between these two groups and should be interpreted with caution. The associations with infertility relative to controls were also significant but considerably weaker, with odds ratios between about 3 and 8.

Table 6: Diagnostic performance of individual biomarkers and the combined six-marker panel for discriminating between groups, derived from receiver-operating-characteristic analysis.

Biomarker	AUC (95% CI)	Cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Testicular cancer vs control						
Galectin-3	0.941 (0.882–0.983)	13.3	83.3	98.3	98.0	85.5
HMGB1	0.916 (0.848–0.973)	4.4	81.7	96.7	96.1	84.1
Survivin	0.933 (0.876–0.982)	92.9	83.3	100.0	100.0	85.7
VEGF	0.951 (0.906–0.986)	341.0	91.7	93.3	93.2	91.8
GDF-15	0.879 (0.806–0.941)	658.5	78.3	91.7	90.4	80.9
Pentraxin-3	0.913 (0.851–0.965)	2.8	81.7	96.7	96.1	84.1
Combined 6-marker panel	**0.989 (0.976–0.998)**	—	**91.7**	**100.0**	—	—
Infertile vs control						
Galectin-3	0.867 (0.797–0.930)	10.6	80.0	83.3	82.8	80.6
HMGB1	0.870 (0.789–0.939)	3.9	80.0	93.3	92.3	82.4
Survivin	0.801 (0.713–0.882)	76.5	66.7	90.0	87.0	73.0
VEGF	0.770 (0.678–0.854)	360.6	56.7	93.3	89.5	68.3
GDF-15	0.761 (0.673–0.841)	562.8	70.0	73.3	72.4	71.0
Pentraxin-3	0.810 (0.728–0.885)	2.6	65.0	90.0	86.7	72.0
Combined 6-marker panel	**0.948 (0.901–0.987)**	—	**88.3**	**93.3**	—	—
Testicular cancer vs infertile						
Galectin-3	0.715 (0.621–0.801)	17.2	53.3	85.0	78.0	64.6
HMGB1	0.711 (0.613–0.807)	7.6	48.3	91.7	85.3	64.0
Survivin	0.789 (0.705–0.870)	133.8	58.3	90.0	85.4	68.4
VEGF	0.782 (0.701–0.859)	471.0	56.7	85.0	79.1	66.2
GDF-15	0.724 (0.632–0.811)	887.8	50.0	88.3	81.1	63.9
Pentraxin-3	0.702 (0.605–0.788)	5.0	50.0	86.7	78.9	63.4
Combined 6-marker panel	**0.863 (0.796–0.928)**	—	**75.0**	**90.0**	—	—

Cut-off values were determined by maximising the Youden index; units are ng/mL for galectin-3 and HMGB1 and pg/mL for the remaining markers. 95% confidence intervals for the AUC were obtained by bootstrap resampling (2000 iterations).

For the combined six-marker panel, predicted probabilities were generated by multivariable logistic regression with stratified five-fold cross-validation to limit optimistic bias. AUC, area under the ROC curve; CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value; TC, testicular cancer.

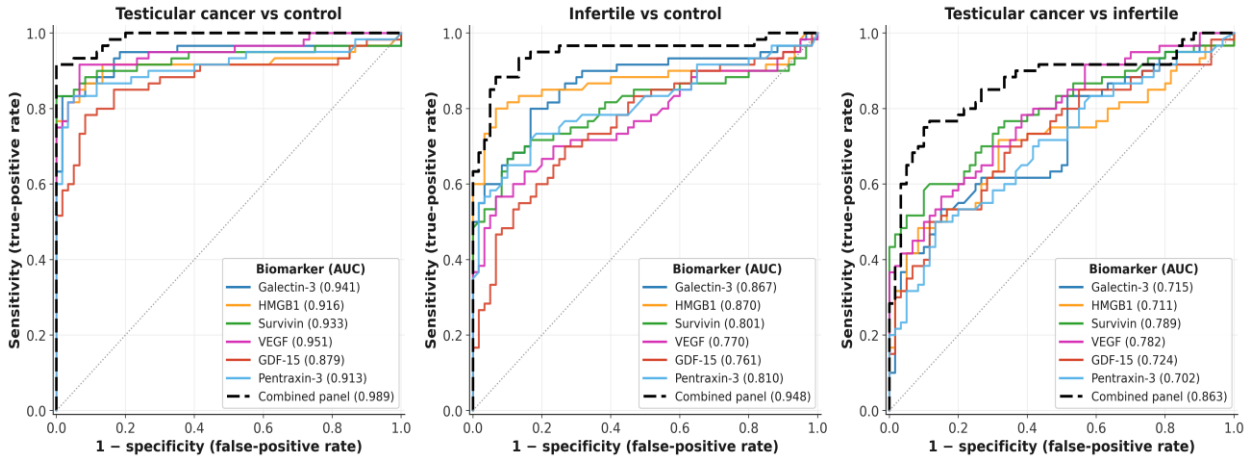

Figure 2: Receiver-operating-characteristic (ROC) curves for the six biomarkers and the combined panel, for the three pairwise contrasts: testicular cancer versus control, infertile versus control, and testicular cancer versus infertile. Coloured lines represent individual biomarkers and the dashed black line the combined six-marker panel (cross-validated logistic-regression probability). The area under the curve (AUC) for each marker is given in parentheses in the legend; the diagonal dotted line indicates no discrimination (AUC = 0.5).

Table 7: Univariable logistic regression: association of each biomarker (per one-standard-deviation increase) with disease status.

Biomarker	TC vs control: OR (95% CI)	P	Infertile vs control: OR (95% CI)	P
Galectin-3	34.32 (9.36–125.82)	<0.001	7.45 (3.65–15.22)	<0.001
HMGB1	24.88 (7.55–81.98)	<0.001	7.95 (3.80–16.63)	<0.001
Survivin	33.28 (9.43–117.46)	<0.001	4.23 (2.43–7.36)	<0.001
VEGF	47.45 (11.42–197.17)	<0.001	3.13 (1.94–5.06)	<0.001
GDF-15	11.97 (4.82–29.76)	<0.001	3.09 (1.87–5.11)	<0.001
Pentraxin-3	54.44 (11.57–256.05)	<0.001	5.59 (2.82–11.10)	<0.001

Odds ratios express the increase in odds of belonging to the disease group per one-standard-deviation increment in biomarker concentration; each biomarker was modelled separately (univariable). The wide confidence intervals for the testicular-cancer contrast reflect near-complete separation between groups and should be interpreted cautiously.

CI, confidence interval; OR, odds ratio; TC, testicular cancer. A two-sided P < 0.05 was considered statistically significant.

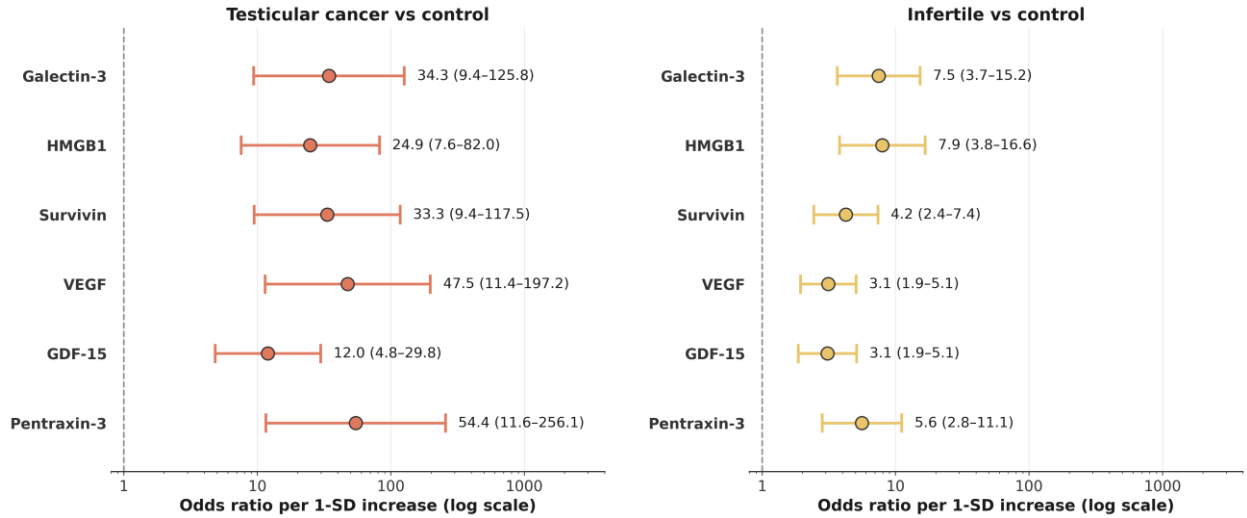

Figure 4: Forest plot of biomarker associations with disease status from univariable logistic regression. Points represent the odds ratio (OR) per one-standard-deviation increase in each biomarker, with horizontal lines indicating 95% confidence intervals (shown numerically to the right of each estimate). Left panel, testicular cancer versus control; right panel, infertile versus control. The dashed vertical line marks the null value (OR = 1); the x-axis is on a logarithmic scale.

DISCUSSION

In this cross-sectional study of 180 men, six circulating proteins spanning inflammation, apoptosis inhibition and angiogenesis, namely galectin-3, HMGB1, survivin, VEGF, GDF-15 and pentraxin-3, were all elevated in a graded fashion from healthy fertile controls, through infertile men, to patients with testicular cancer. The increments between adjacent groups were statistically robust for every marker, the markers correlated with conventional semen and hormonal parameters in a biologically coherent direction, and a combined six-marker panel separated the clinical groups with good accuracy. To our knowledge, this is the first study to evaluate these particular six biomarkers together across these three groups, and the first to do so in an Iraqi population.

The progressive elevation across the reproductive-health spectrum fits the prevailing biological understanding of these conditions. Male infertility and testicular germ-cell tumours are connected developmentally through the testicular dysgenesis syndrome and epidemiologically through an approximately two-fold increased risk of testicular cancer among infertile men.[3,4,5,6] A shared feature of both states is a disturbed testicular micro-environment marked by oxidative stress, low-grade inflammation and dysregulated cell turnover, and the stepwise rise we observed in C-reactive protein paralleled the biomarker gradient, supporting the view that systemic inflammation intensifies along this continuum. Each marker has a plausible mechanistic role. Galectin-3 modulates inflammation, fibrosis and apoptosis and is released into the circulation during tissue injury;[8] its elevation in azoospermic men and inverse relationship with testosterone have been reported previously,[9] and in our data it showed both the highest concentrations relative to controls and the strongest correlations with semen quality and follicle-stimulating hormone. HMGB1, acting as a damage-associated molecular pattern, drives germ-cell apoptosis by shifting the Bcl-2/Bax balance and sustains testicular inflammation, and its blockade mitigates experimental orchitis;[10,11,12] the parallel rise of HMGB1 and C-reactive protein in our cohort is consistent with this inflammatory mechanism. Survivin links the two conditions from opposite directions: it is required for the normal balance of germ-cell proliferation and apoptosis, yet it is strongly re-expressed in testicular germ-cell tumours, where it blocks apoptosis,[13] which may explain why it showed the largest effect size and the highest concentrations in the cancer group. VEGF underlies both the abnormal angiogenesis of the infertile testis, as seen in varicocele,[18] and tumour neovascularisation, and accordingly produced the highest single-marker discrimination between cancer and controls. GDF-15 is induced by oxidative stress, inflammation and endothelial damage and functions as a tumour-associated biomarker and immune checkpoint;[14,15] its more modest gradient and effect size in our data are in keeping with the observation that circulating GDF-15 in testicular cancer rises most clearly in the context of treatment-related endothelial injury rather than at baseline.[15] Pentraxin-3, a long pentraxin bridging innate immunity and reproduction, is associated with poorer survival across malignancies,[16,17] and behaved here as a sensitive inflammatory marker that rose steeply in the cancer group.

The biomarker correlations with semen and hormonal parameters add internal coherence to these findings. The consistent inverse relationship with sperm concentration, motility, morphology and vitality, the inverse relationship with inhibin B, and the direct relationship with follicle-stimulating hormone together describe a pattern in which higher biomarker concentrations accompany more severe spermatogenic failure. Galectin-3 and HMGB1 showed the strongest of these associations, which is consistent with their direct involvement in germ-cell apoptosis and Sertoli-cell dysfunction. We emphasise, however, that these are correlations in a cross-sectional sample and cannot establish whether the biomarkers contribute to, or merely reflect, impaired spermatogenesis.

Our results can be placed alongside other work from Iraq. Faris and colleagues, studying infertile men in Najaf, documented the same hierarchy of impaired semen quality and disturbed hormonal indices that we observed, underscoring the high local burden of male-factor infertility and the value of laboratory markers beyond the conventional semen analysis.[24] Khadhim and Manshd identified circulating microRNAs as candidate biomarkers of idiopathic male infertility in an Iraqi cohort, illustrating a parallel interest in minimally invasive serum markers,[25] and Kadhim and colleagues reported that telomere length and mitochondrial DNA copy number distinguished infertile from fertile Iraqi men, again pointing to molecular signatures of testicular dysfunction.[26] Methodologically, our serum ELISA approach follows an established local tradition of quantifying serum immune-inflammatory proteins by sandwich ELISA in Najaf research laboratories.[27] Our study extends this body of work by adding a panel of inflammatory, apoptotic and angiogenic proteins and, importantly, by including a testicular cancer group, which previous Iraqi studies of male infertility biomarkers did not examine.

The findings are also consistent with international data on the individual markers. Elevated serum galectin-3 in azoospermia,[9] the pro-apoptotic and pro-inflammatory actions of HMGB1 in the testis,[10,11,12] survivin over-expression in testicular germ-cell tumours,[13] the role of VEGF in varicocele and tumour angiogenesis,[18] and the prognostic value of pentraxin-3 across cancers[17] have each been described separately. Our contribution is to show that, when measured together in the same individuals, these markers form a coherent gradient and that their combination discriminates better than any single protein. The very high cross-validated accuracy of the combined panel for separating cancer from controls (area under the curve 0.989) should nonetheless be read with caution: the two groups differ in many respects, the confidence intervals on several odds ratios were extremely wide because of near-complete separation, and such performance in a single sample typically attenuates on external validation.

These observations carry potential clinical implications, but only as hypotheses. A serum panel of this kind is attractive because it is minimally invasive and inexpensive relative to imaging or tissue sampling, and because it might, in principle, help flag infertile men who merit closer surveillance for testicular pathology, given their established excess risk. The markers are not tumour-specific, however, and the modest separation between the infertile and cancer groups (combined area under the curve 0.863) makes clear that the panel could not, on these data, replace established diagnostic pathways such as scrotal ultrasonography, conventional serum tumour markers and histology, nor the more specific emerging markers such as microRNA-371a-3p.[7] Any clinical role would therefore be adjunctive and would need to be defined through prospective study.

The study has several strengths. The three groups were of equal size and well matched for age and major lifestyle factors, including smoking; all six biomarkers were measured under identical, blinded conditions; the analysis combined omnibus testing with effect sizes, variance-appropriate post-hoc methods, correlation analysis, receiver-operating-characteristic analysis and cross-validated modelling; and the inclusion of three groups allowed the markers to be examined across a clinically meaningful spectrum rather than in a single case-control contrast. The limitations are equally important. The design was cross-sectional and single-centre, so no causal or temporal inference is possible and the generalisability beyond this setting is uncertain. The sample size, although adequate for the primary comparisons, was modest for the diagnostic-modelling analyses, and internal cross-validation does not substitute for external validation; the combined-panel estimates in particular are likely optimistic. Blood from cancer patients was obtained before systemic therapy but sometimes after orchidectomy, which could itself influence circulating markers. We did not measure these proteins longitudinally or after treatment, did not include men with other benign scrotal or inflammatory conditions that might also raise the markers, and did not adjust the per-marker odds ratios for the strong inter-correlation among the biomarkers. Finally, the markers are biologically non-specific, reflecting inflammation, apoptosis and angiogenesis common to many disorders.

Several directions follow from this work. The panel should be tested in larger, multi-centre cohorts with external validation and, ideally, in prospective designs that follow infertile men over time to determine whether baseline biomarker concentrations predict the later development of testicular pathology. Comparison groups should be broadened to include men with varicocele, orchitis and other benign conditions in order to establish specificity, and the panel should be benchmarked against, and potentially combined with, established and emerging tumour markers. Longitudinal measurement before and after treatment would clarify whether the markers track disease activity. Until such data exist, the present findings are best regarded as a biologically coherent, internally consistent description of how these six circulating proteins behave across the male reproductive-health spectrum, rather than as evidence for a clinical test.

CONCLUSION

In this single-centre, cross-sectional study, serum galectin-3, HMGB1, survivin, VEGF, GDF-15 and pentraxin-3 were graded across healthy fertile controls, infertile men and patients with testicular cancer, increasing progressively across the three groups and correlating with conventional semen and hormonal parameters in a biologically coherent direction. A combined six-marker panel discriminated the groups with good accuracy, most clearly between testicular cancer and controls and least between testicular cancer and infertile men. These findings identify a coherent panel of inflammatory, apoptotic and angiogenic markers that merits further study, but they are hypothesis-generating; the markers are not disease-specific, and external, prospective validation is required before any clinical application can be considered.

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