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Molecular and biochemical profile of testicular germ cell tumors: Diagnostic implications

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Abstract

Testicular germ cell tumors (TGCTs) require a precise multimodal diagnostic framework. This study evaluated the diagnostic performance and correlation of histopathology, immunohistochemistry (IHC), and serum biomarkers across tumor subtypes. Sixty male participants were enrolled: 40 patients with histologically and immunophenotypically confirmed primary testicular neoplasia and 20 healthy controls. Serum β -hCG, AFP, and LDH were quantified, and tissue sections were analyzed using a targeted IHC antibody panel (OCT3/4, PLAP, CD117, CD30, Ki-67). Pure seminoma was the most frequent variant (55.0%, *n* = 22), followed by non-seminomatous germ cell tumors (NSGCTs; 42.5%, *n* = 17) and sex cord-stromal tumors (2.5%, *n* = 1). Serum AFP elevation was found in 58.8% of NSGCTs but was rare in seminomas (9.1%), while β -hCG elevation was more prevalent in NSGCTs (52.9%) than in seminomas (31.8%). Combined β-hCG and AFP positivity occurred in 47.1% of NSGCTs compared to 4.5% of seminomas. Tissue-based IHC expression patterns strongly validated serological marker behaviors and subtype boundaries. Synchronized clinical, serological, and immunohistochemical profiling minimizes diagnostic ambiguity, optimizes subtype classification, and reinforces risk-adapted therapeutic management for young adult patients.

Introduction

Testicular germ cell tumors (TGCTs) are the most common malignancies affecting young adult men and account for approximately 95% of primary testicular cancers [1–3]. These tumors comprise a heterogeneous group of neoplasms characterized by distinct histopathological features, variable clinical behavior, and different responses to therapy. The incidence of TGCTs varies considerably worldwide, with the highest rates reported in Northern European countries and lower rates observed in Asian and African populations [24–26]. Several factors, including genetic susceptibility, environmental exposures, endocrine-disrupting agents, and perinatal influences, have been associated with TGCT development [27–29]. Recent genetic studies suggest that inherited factors contribute substantially to disease susceptibility, particularly through genes involved in germ cell differentiation, testicular development, and hormonal regulation [30]. Current evidence indicates that most TGCTs originate from germ cell neoplasia in situ (GCNIS), a precursor lesion characterized by impaired germ cell maturation and abnormal retention of embryonic features [13–16]. Molecular studies have identified several alterations associated with tumor development and progression, including chromosome 12p amplification, dysregulation of KIT signaling pathways, and altered expression of pluripotency-associated transcription factors such as NANOG, SOX17, and OCT3/4 [17–20]. Mechanistically, seminomatous and non-seminomatous germ cell tumors (NSGCTs) demonstrate distinct molecular and epigenetic profiles that contribute to differences in tumor biology and therapeutic response [21–23]. Clinically, early diagnosis remains essential because prognosis and treatment outcomes are strongly associated with disease stage. Seminomas generally show slower progression and high sensitivity to radiotherapy and platinum-based chemotherapy, whereas NSGCTs often demonstrate more aggressive behavior and earlier metastatic spread [31–33]. Certain tumor subtypes may also produce elevated serum β-human chorionic gonadotropin (β-hCG), leading to endocrine manifestations such as gynecomastia [34, 35]. Although imaging techniques,

including ultrasonography, computed tomography (CT), and magnetic resonance imaging (MRI), are important for clinical evaluation and staging, imaging findings alone are insufficient for definitive diagnosis, making histopathological confirmation essential [40, 41]. Accurate diagnosis and classification of TGCTs require an integrated diagnostic approach that combines histopathology, immunohistochemistry (IHC), and serum tumor biomarkers [4, 5]. Histopathological examination remains the gold standard for tumor classification and for distinguishing seminomatous from non-seminomatous components [5]. Immunohistochemical markers such as OCT3/4, PLAP, CD117, CD30, SALL4, SOX17, SOX2, glypican-3, and Ki-67 are widely used to improve diagnostic accuracy and to identify mixed histological patterns [6, 7, 42–45]. Recent World Health Organization (WHO) classifications and international consensus recommendations also emphasize the importance of GCNIS identification and standardized immunophenotypic characterization in the evaluation of TGCTs [46–48]. Beyond tissue-based diagnostics, serum biomarkers including β-hCG, alpha-fetoprotein (AFP), and lactate dehydrogenase (LDH) play an important role in staging, treatment monitoring, and detection of disease recurrence [8–10, 36–39]. International clinical guidelines from organizations such as the EAU, ESMO, NCCN, and IGCCCG recommend integrating clinical, radiological, histopathological, immunophenotypic, and biochemical findings for optimal patient management [18, 40, 49]. This multidisciplinary approach supports accurate risk stratification and individualized therapeutic planning for patients with TGCTs [49, 50]. Despite advances in diagnostic protocols, variations in biomarker expression and histopathological patterns continue to present diagnostic challenges in routine clinical practice. Furthermore, regional differences among patient populations highlight the importance of validating diagnostic findings across different clinical settings and cohorts. To address this, the aim of this study was to evaluate the diagnostic value and correlation of histopathological findings, immunohistochemical markers, and serum biomarkers (β-hCG, AFP, and LDH) across different subtypes of testicular germ cell tumors. By integrating these parameters, this study establishes a correlated diagnostic matrix to improve classification accuracy and guide risk-adapted clinical management for patients presenting with TGCTs.

Materials and Methods

Study Design and Participant Selection

This prospective observational study was conducted at the Clinic of Medical Biochemistry and the Pathology Service of the University Clinical Center of Kosova, Pristina, Kosovo, in collaboration with the private pathology laboratory “Pathology”, Pristina, Kosovo. Ethical approval for the study was obtained from the institutional Ethics Committee, and all participants provided written informed consent prior to inclusion in accordance with the principles of the Declaration of Helsinki. A total of 60 male participants were evaluated during the study period and divided into two distinct arms. The patient cohort consisted of 40 adult males diagnosed with primary testicular tumors. Lineage diagnosis was established through physical clinical examination, scrotal Doppler ultrasonography, computed tomography (CT) staging, serum tumor marker assessment, and formal histopathological confirmation following radical inguinal

orchiectomy. Patients who underwent prior chemotherapy or radiotherapy before surgical intervention were excluded from enrollment. The control group included 20 age-matched healthy male volunteers without a history of malignant disease, systemic inflammatory disorders, or testicular pathology, serving to establish baseline comparative values for the serum biochemical markers. Clinical profiles, laboratory findings, and histopathological data were systematically recorded for all participants.2.2. Sample Collection and Biochemical AnalysisPeripheral venous blood samples were obtained from all participants during morning hours using serum separation tubes containing clot activator and gel. Samples were centrifuged at 3000 rpm for 10 minutes, and biochemical analyses were completed within 2 hours after collection to reduce pre-analytical variability and minimize sample hemolysis.Serum β-human chorionic gonadotropin (β-hCG) and alpha-fetoprotein (AFP) concentrations were quantified using the Cobas e 411 automated analyzer (Roche Diagnostics, Basel, Switzerland) via electrochemiluminescence immunoassay (ECLIA) technology. Calibration and analytical runs followed the manufacturer's instructions. Serum lactate dehydrogenase (LDH) activity was determined using a kinetic spectrophotometric method on the ILAB Taurus chemistry analyzer (Instrumentation Laboratory, Bedford, MA, USA), measuring the conversion of lactate to pyruvate at a wavelength of 340 nm. Internal quality control procedures were performed routinely to ensure analytical accuracy and reproducibility. β-hCG values were expressed in IU/L, AFP in ng/mL, and LDH in U/L.2.3. Histopathological Evaluation Following radical orchiectomy, tissue specimens were fixed in 10% neutral buffered formalin for 24–48 hours and subsequently processed using standard paraffin-embedding techniques. Tissue sections measuring 4 μm in thickness were prepared and stained with hematoxylin and eosin (H&E) for routine light microscopic evaluation. Histopathological classification followed the current World Health Organization (WHO) criteria. Tumors were categorized as seminoma, non-seminomatous germ cell tumor (NSGCT), or mixed germ cell tumor. Non-seminomatous tumors included embryonal carcinoma, yolk sac tumor, choriocarcinoma, and teratoma components.

Immunohistochemical Profiling

Immunohistochemical (IHC) analysis was performed on representative tumor sections to confirm specific lineages and support definitive histopathological classification. The antibody panel utilized for differentiation consisted of OCT3/4, placental alkaline phosphatase (PLAP), CD117 (c-KIT), CD30, AFP, hCG, and Ki-67. Mechanistically, OCT3/4 and PLAP served for generalized germ cell tumor identification, whereas CD117 expression was primarily evaluated in seminoma cases. CD30 was assessed to isolate embryonal carcinoma components, whereas AFP and hCG staining supported the identification of yolk sac and trophoblastic differentiation, respectively. Ki-67 immunostaining was applied to quantify active tumor proliferative activity. All stained slides were independently reviewed by two experienced pathologists, and representative microscopic findings were documented using digital imaging.

Statistical Analysis

Statistical processing of the data was performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA) and Microsoft Excel. Data distribution was evaluated using the Kolmogorov–Smirnov test. Normally distributed continuous variables were expressed as mean ± standard deviation (SD) and compared between groups using the independent-samples Student's t-test. Continuous variables displaying a non-normal distribution were expressed as medians with interquartile ranges (IQR) and analyzed using the non-parametric Mann-Whitney U test. Categorical variables were evaluated using the Chi-square (X^2) test or Fisher's exact test where appropriate. A *p*-value below 0.05 ($p < 0.05$) was considered statistically significant.

Results

Study Population

The study cohort comprised a total of 60 male participants, which included 40 patients presenting with histopathologically confirmed primary testicular malignancies and 20 age-matched healthy control subjects. All biological specimens were systematically collected, processed, and evaluated according to standardized laboratory quality protocols. No significant pre-analytical variations or analytical abnormalities were identified during sample processing or subsequent biochemical quantification.

Patient Age Profiles

Stratification of the patient cohort by age intervals showed that the highest incidence of primary testicular malignancies occurred within the 25–29 years age cohort (20.0%, *n* = 8), followed by the 30–34 years group (17.5%, *n* = 7) and the 35–39 years interval (15.0%, *n* = 6). A lower frequency of cases was registered at the extremes of the age spectrum, with patients aged 1–19 years accounting for 5.0% (*n* = 2) and those aged older than 50 years accounting for 12.5% (*n* = 5). Overall, these findings demonstrated a peak disease presentation occurring during the third decade of life (Table 1).

Table 1
Age distribution of patients presenting with primary testicular tumors (*n* = 40)

Age group (years)	Number of Cases (*n*)	Percentage %
1–19	2	5.0
20–24	5	12.5
25–29	8	20.0
30–34	7	17.5
35–39	6	15.0
40–44	4	10.0
45–49	3	7.5
≥ 50	5	12.5
Total	**40**	**100.0**

Histopathological Subtype Presentation

Based on definitive histopathological classification of the surgical specimens, pure seminoma was identified as the most frequent tumor type, accounting for 55.0% (*n* = 22) of the patient cohort, followed by non-seminomatous germ cell tumors (NSGCTs) at 42.5% (*n* = 17). Sex cord-stromal tumors represented a rare component, accounting for 2.5% (*n* = 1) of total evaluated cases (Table 2).

Table 2
Histopathological distribution of primary testicular tumor types (*n* = 40).

Tumor type	Number of cases (*n*)	Percentage %
Seminoma	22	55.0
NSGCT	17	42.5
Sex Cord-Stromal Tumor	1	2.5
Total	**40**	**100.0**

Individual Serum Tumor Marker Elevations

The serum expression patterns for β-hCG, AFP, and LDH were mapped across all evaluated clinical cohorts (Table 3). Elevation of β-hCG was more frequent in the NSGCT cohort (52.9%, 9/17) compared to the seminoma group (31.8%, 7/22). Serum AFP elevation occurred predominantly within the NSGCT group (58.8%, 10/17) and was uncommon in pure seminomas (9.1%, 2/22). Total LDH activity showed

comparable frequency elevations between the two malignant cohorts, being elevated in 36.4% (8/22) of seminomas and 41.2% (7/17) of NSGCT cases. In the healthy control group, serum β-hCG and AFP levels remained completely within standard physiological reference limits for all participants (*n* = 20), whereas isolated LDH elevation was registered in two individuals (10.0%) due to documented benign, non-neoplastic musculoskeletal conditions.

Table 3
Frequency and distribution of elevated serum tumor markers across cohorts.

Clinical Cohort (*N*)	**Normal β-hCG (*n*)**	**Elevated β-hCG, *n* (%)**	**Normal AFP (*n*)**	**Elevated AFP, *n* (%)**	**Normal LDH (*n*)**	**Elevated LDH, *n*(%)**
Seminoma (*n* = 22)	15	7 (31.8%)	20	2 (9.1%)	14	8 (36.4%)
NSGCT (*n* = 17)	8	9 (52.9%)	7	10 (58.8%)	10	7 (41.2%)
Sex Cord-Stromal (*n* = 1)	1	0 (0.0%)	1	0 (0.0%)	0	1(100.0%)
Healthy Controls (*n* = 20)	20	0 (0.0%)	20	0 (0.0%)	18	2 (10.0%)

Concurrent Multi-Marker Profiles

Biochemical phenotypes were assessed through combined multi-marker serology tracking (Table 4). Simultaneous elevation of serum β-hCG and AFP was observed in 4.5% (*n* = 1) of pure seminomas in contrast to 47.1% (*n* = 8) of the NSGCT cases. Co-elevation of β-hCG and LDH was found in 13.6% (*n* = 3) of the seminoma group and 17.6% (*n* = 3) of the NSGCT group. Combined AFP and LDH positivity was noted in 4.5% (*n* = 1) and 23.5% (*n* = 4) of the cohorts, respectively. Triple-marker elevation involving simultaneous serum peaks of β-hCG, AFP, and LDH was rare in pure seminomas (4.5%, *n* = 1) but was confirmed in 11.8% (*n* = 2) of patients diagnosed with NSGCTs. Multi-marker cross-elevations were entirely absent within the healthy control arm.

Table 4
Multi-marker co-elevation profiles across distinct diagnostic cohorts.

Tumor Type	Elevated β-hCG & AFP	Elevated β-hCG & LDH	Elevated AFP & LDH	Triple Elevated (β-hCG, AFP & LDH)
Seminoma (*n* = 22)	1 (4.5%)	3 (13.6%)	1 (4.5%)	1 (4.5%)
NSGCT (n = 17)	8 (47.1%)	3 (17.6%)	4 (23.5%)	2 (11.8%)
Sex Cord-Stromal (*n* = 1)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Healthy Controls (*n* = 20)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

Immunohistochemical Profiling

Immunohistochemical expression analyses validated the baseline morphologic classifications. Pure seminomas demonstrated intense, diffuse membranous and nuclear reactivity patterns for OCT3/4, PLAP, and CD117, confirming their uncommitted classical germ cell lineage. Conversely, NSGCT specimens displayed varied, subtype-specific antigen profiles, showing strong selective reactivity for CD30 in areas of embryonal carcinoma, focal intracellular staining for AFP in component regions of yolk sac tumors, and cytoplasmic positivity for hCG within syncytiotrophoblastic cells. Assessment of the Ki-67 nuclear antigen showed high proliferative fractions across all malignant tissue samples, with the highest labeling indexes systematically identified within non-seminomatous sheets. Mixed germ cell malignancies were characterized by distinct regional variations matching their specific cellular components, allowing for precise tissue segment partitioning.

Discussion

This study establishes an integrated clinicopathological and biochemical evaluation of testicular germ cell tumors (TGCTs) within a regional patient cohort from Kosovo. The findings highlight the clinical utility of correlating systemic serum tumor marker dynamics directly with tissue-based histopathology and targeted immunohistochemical (IHC) profiling for the comprehensive diagnostic characterization of TGCTs. Furthermore, the demonstrated alignment between these distinct serological and morphological parameters supports the diagnostic value of a multimodal clinical approach, which remains essential for achieving precise tumor classification and definitive subtype differentiation.

Age Distribution and Histological Specification

The identified disease concentration within the third decade of life, showing a peak frequency of 20.0% in the 25–29 years cohort and 17.5% in the 30–34 years cohort, is congruent with established global epidemiological behavior. Large population-based registers confirm that TGCTs represent the premier malignancy affecting young adult males, with an incidence apex typically spanning ages 20 to 34 [3, 51].

Within our prospective cohort, pure seminoma was identified as the predominant histological variant 55.0% compared to non-seminomatous germ cell tumors 42.5%, a ratio distribution that matches contemporary European pathological reports where classical seminomas consistently represent the slight majority of primary testicular neoplasms [1, 52].

Diagnostic Sensitivity of Individual Serological Markers

MarkersOur quantitative serological metrics validate the established clinical diagnostic roles of serum β -hCG, AFP, and LDH in tracking TGCT variations. The marked difference in serum AFP elevations-noted in 58.8% of the NSGCT group compared to just 9.1% within pure seminomas-is consistent with international clinical guidelines specifying that yolk sac or embryonal carcinoma components actively generate AFP, while pure seminomas remain fundamentally non-productive for this oncofetal antigen [53]. Similarly, the higher prevalence of serum β -hCG peaks within the non-seminomatous cohort 52.9%\ over seminomas 31.8% matches documented clinical data showing increased systemic hormone circulation driven by advanced choriocarcinomatous elements or isolated syncytiotrophoblastic giant cells [54]. In contrast, total LDH activity demonstrated closely balanced elevations across both the seminoma 36.4%\ and NSGCT 41.2% subsets in our study cohort. This pattern confirms the status of LDH as a non-specific indicator reflecting overall tumor burden, tissue turnover, and total cell mass rather than a localized discriminator of specific histological subtypes. This finding is highly concordant with the clinical analyses of Gilligan et al. and the European Association of Urology, which characterize serum LDH performance strictly as a prognostic gauge rather than a subtype-specific diagnostic index.

Combined Marker Co-Elevations and Biochemical

PhenotypingA primary focus of this investigation was tracking concurrent multi-marker serological peaks to map multi-variant biochemical phenotypes. The significant elevation of simultaneous serum β-hCG and AFP in 47.1% of NSGCT cases compared to a minimal 4.5% within pure seminoma specimens underscores the diagnostic power of synchronized multi-marker screening over single-antigen baseline evaluation. These clinical measurements align directly with large retrospective series indicating that comprehensive multi-marker screening strategies significantly improve overall baseline subtype discrimination and clinical staging accuracy [53, 55]. Furthermore, the detection of triple-marker peaks (co-elevating β-hCG, AFP, and LDH) almost exclusively in the non-seminomatous cohort (11.8% vs. 4.5% in seminoma) emphasizes that highly dynamic serum index balances reflect advanced cellular heterogeneity and aggressive underlying tumor biology, traits commonly linked to higher risk stratifications in international consensus guidelines [56].

Immunohistochemical Correlation with Biochemical Findings

Our tissue-based immunohistochemical profiling data strongly reinforce the overall diagnostic accuracy of the definitive histopathological classification framework. The dense nuclear and membranous reactivity observed for OCT3/4, PLAP, and CD117 within the pure seminoma cases, contrasted against the selective expression of CD30, AFP, and cytoplasmic hCG within the non-seminomatous sheets,

closely parallels the standard diagnostic antigen panels recommended in contemporary pathology references [57, 58]. Furthermore, the precise identification and architectural mapping of mixed germ cell malignancies using these specific IHC lines within our prospective series confirms previous diagnostic reports that systematic immunophenotyping is indispensable for detecting minor, occult tumor components [11]. Identifying these hidden malignant segments is critical, as they alter formal baseline staging indices and directly impact immediate downstream oncological treatment decisions [11].

Clinical and Diagnostic Implications

The strong concordance identified between our serological, histopathological, and immunohistochemical findings underscores the fundamental importance of a multimodal diagnostic strategy, a consolidated framework explicitly recommended by major international clinical guidelines including the EAU, ESMO, and NCCN [18, 40, 49]. Our baseline results support the routine clinical tracking of serum AFP and β-hCG kinetics for accurate subtype differentiation, while confirming the utilization of total LDH activity as a reliable systemic indicator of overall tumor burden and prognosis rather than lineage specificity. Crucially, the absolute absence of β-hCG and AFP elevations within our healthy control arm, contrasted against isolated, transient LDH increases noted in two control individuals, reinforces the high organ-specific diagnostic specificity of these biomarkers when evaluated within an appropriate clinical context, a standard repeatedly emphasized in contemporary biomarker-focused reviews [12, 59].

Strengths and Limitations

A major strength of this investigation lies in its prospective, integrated diagnostic design, which systematically pairs real-time serological blood marker concentrations with matching tissue biopsies and standardized immunohistochemical profiling. This comprehensive approach aligns directly with contemporary gold-standard protocols for testicular germ cell tumor management. Furthermore, this study contributes original clinical and laboratory datasets from a specific Western Balkan population that is currently underrepresented in international oncological registries. Despite these clear methodological strengths, several limitations must be acknowledged. First, the study is constrained by a relatively moderate cohort size consisting of forty primary tumors, which reflects the single-center setting. Second, our baseline laboratory setup lacked extended molecular sequencing capabilities, such as micro RNA-371a-3p tracking or genomic copy-number variations. Finally, this cohort lacks long-term post-orchiectomy clinical follow-up data, preventing the formal correlation of these multi-marker profiles with definitive recurrence-free survival timelines. Future multicenter initiatives incorporating advanced genomic or epigenetic profiling are warranted to further refine local risk stratification indices and support personalized therapeutic pathways.

Summary

The clinical observations compiled throughout this study are in strong agreement with high-quality, Scopus- and Web of Science (WoS)-indexed oncology literature, validating that the synchronized utilization of serum tumor markers, interpreted strictly in conjunction with baseline histopathology and

standardized immunohistochemical validation, provides the most reliable diagnostic framework for managing patients with TGCTs [11, 12]. This integrated multi-modal strategy substantially improves overall subtype differentiation, supports more accurate real-time clinical staging, and carries direct operational implications for deploying individualized, risk-adapted therapeutic monitoring pipelines in contemporary clinical practice.

Conclusions

In conclusion, this prospective study confirms that the integrated application of serum tumor markers, evaluated alongside structured histopathological classification and standardized immunohistochemical panels, provides a highly specific and reliable diagnostic matrix for testicular germ cell tumors within the regional population of Kosovo. The individual and combined distribution profiles of serum β-hCG, AFP, and LDH align with established global clinical standards, effectively separating classical seminomas from aggressive non-seminomatous lineages. This multi-method validation supports precise tumor subtyping and diagnostic accuracy within local clinical pipelines, establishing a robust baseline for risk-adapted therapeutic deployment and long-term clinical monitoring protocols in young adult male patients.

Declarations

Ethics declarations

Informed consent.

Informed consent was obtained from all subjects involved in the study.

Competing interests

The authors declare no competing interests.

Ethics approval

Ethical approval was obtained from the institutional Ethics Committee of the Kosovo Doctors Chamber nr 9923.

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Author Contribution

L.S. and V.H. conceptualized the study. L.S., V.H., and K.K. conducted the investigation. M.G. managed the software. K.K., L.S., and I.H. prepared the original draft. L.S., I.H., M.G., S.U., and E.K. reviewed and edited the manuscript. E.K. handled the visualization. All authors have read and approved the final manuscript.

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Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request

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