Germ cell immunoprofile and KIT exon 17 mutation guide rectification of seminoma misclassified as epithelioid gastrointestinal stromal tumor: A case report

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Received June 3, 2025; Accepted October 9, 2025

DOI: 10.3892/br.2025.2088

Abstract. Seminoma, derived from germ cell neoplasia *in situ*, is the most common testicular germ cell neoplasm. Seminoma exhibits overlapping genetic mutations and immunohistochemical profiles with diverse tumors, substantially complicating diagnosis, particularly in biopsy specimens. In the present study a patient was reported, harboring a huge abdominal mass, who was initially diagnosed as an epithelioid gastrointestinal stromal tumor via biopsy pathology due to CD117 immunohistochemical expression and detection of KIT mutation in exon 17 (p.Y823D). After first-to fourth-line targeted therapies, the patient exhibited rapid tumor progression, promoting diagnostic reevaluation. Definitive diagnosis of seminoma was established through expanded immunohistochemistry (OCT4+/SALL4+/PLAP+/D2-40+) and molecular confirmation of the characteristic KIT p.D816Y mutation, which concurrently explains the observed imatinib resistance. In conclusion, the findings of the present study highlighted the imperative of integrating morphology, immunohistochemistry and context-specific molecular profiling to avoid diagnostic in CD117-positive tumors.

Introduction

Seminoma, comprising 55% of all testicular malignancies, is the most common subtype of testicular cancer (1). The widely accepted hypothesis for the origin of testicular germ cell tumors (TGCT) is that they derive from primordial germ cell or gonocytes whose maturation is disturbed. Seminoma usually occurs in patients ranging from 15 to 44 years of age (2). It is reported that epidemiological risk factors of TGCT include previous TGCT in the contralateral testis, cryptorchidism, hypospadias, male infertility and exposure to environment factors, such as organochlorines, polychlorinated biphenyls, polyvinyl chloride, phthalates, cannabis and tobacco (3). A primary abdominal seminoma harboring KIT mutation was reported, which was misdiagnosed as gastrointestinal stromal tumor via biopsy pathology, revealing the diagnostic pitfalls caused by atypical pathological features, insufficient immunohistochemical data and inadequate understanding of the KIT mutation-associated disease spectrum.

Case presentation

A 53-year-old male patient was admitted to a local hospital due to fatigue in March 2024, with no previous medical history. The 53-year-old male patient was found to have a huge abdominal mass with a maximum diameter of 16 cm, via computed tomography (CT). Ultrasound-guided needle biopsy was performed to establish a definitive histopathological diagnosis prior to multidisciplinary therapeutic planning. Histologically, significantly atypical cells, characterized by nuclei pleomorphism, increased nucleoplasmic ratio, prominent nucleoli, granular chromatin and visible mitotic figures, were observed to be diffusely distributed, with scattered infiltration of inflammatory cells, small vessel formation, and fibrous tissue hyperplasia (Fig. 1). Immunohistochemical staining for CD117, DOG-1, CD34, Des, and S100 was performed using the DAKO immunohistochemical automatic staining machine (DAKO Omnis/GI100) following Envision 2-step protocol. The primary antibodies used were CD117 rabbit anti-human (clone 104D2; 1:100 dilution), Des rabbit anti-human (clone D33; 1:100 dilution), and S100 rabbit anti-human (clone A5109; 1:500 dilution) purchased from Dako; Agilent Technologies, Inc., DOG-1 rabbit anti-human (clone SP31, 1:100 dilution) purchased from Abcam, and CD34 mouse anti-human (clone QBEnd/10, 1:100 dilution) purchased from Shanghai Long Island Antibody Diagnostic Inc. In brief, the tissue sections were subjected to dewaxing and heat-induced antigen retrieval at 95˚C. Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide at 37˚C for 30 min. Antigen retrieval was performed in citrate buffer (10 mmol/l, pH 6.0) at 100°C for 30 min, followed by three 5-minute washes in PBS. The sections were then blocked with 10% normal goat serum [Biorigin

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Key words: seminoma, gastrointestinal stromal tumor, KIT mutation, CD117, D2-40

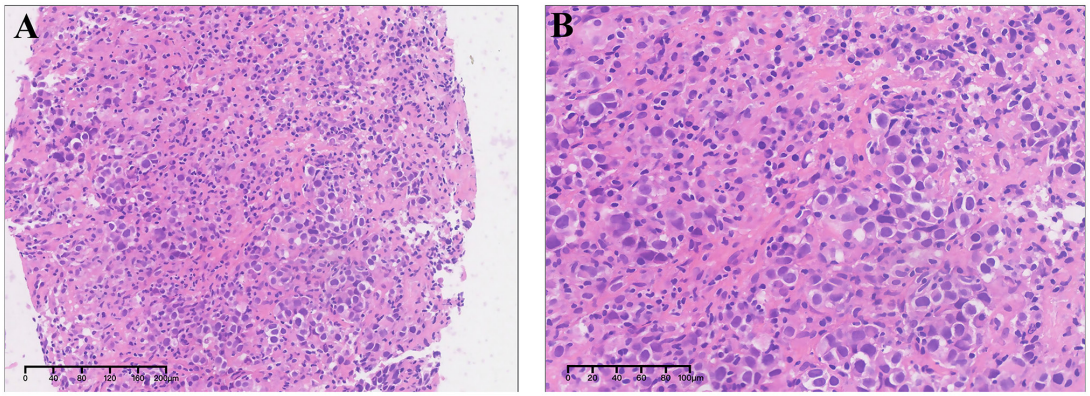

Figure 1. Histological findings in this case. (A) Tumor cells were diffusely distributed, with scattered lymphocyte infiltration. (B) Tumor cells exhibited significant atypia, showing deeply stained nuclei and increased nucleoplasmic ratio.

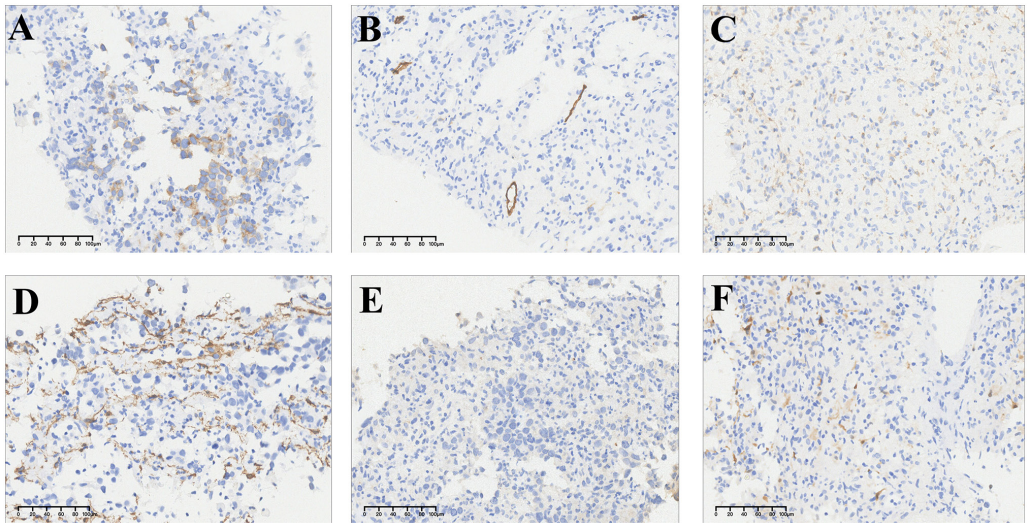

Figure 2. Immunohistochemistry for (A) CD117 (positive), (B) CD34 (negative), (C) DOG-1 (negative), (D) SMA (negative), (E) Des (negative) and (F) S-100 (negative).

(Beijing) Inc.] for 30 min. Subsequently, diluted primary antibodies were applied and incubated overnight at 4˚C. After incubation, Dako Envision kit HRP (cat. no. K4006; Dako; Agilent Technologies, Inc.) was added as a secondary antibody and incubated at 37˚C for 20 min, followed by three PBS washes. Color development was carried out using DAB, and counterstaining was performed with hematoxylin for 30 sec to 1 min. Finally, the sections underwent gradient dehydration, clearing, and mounting with neutral balsam. An Olympus BX43F microscope was used to interpret results. Immunohistochemically, the tumor cells were positive for CD117, but negative for DOG-1, CD34, SMA, Des and S100 (Fig. 2). Amplicon-based targeted next-generation sequencing (NGS) in the affiliated hospital of Jiangnan University detected molecular alternations, revealing the mutation in exon 17 of KIT gene (p.Y823D). A diagnosis of malignant epithelioid GIST was made. Given the massive lesion exceeding 16 cm in maximal diameter, surgical evaluation deemed the tumor unresectable for radical resection at the external institution, prompting attempted targeted therapy to assess potential tumor downsizing for further surgical intervention. Then the patient received daily oral imatinib 400 mg. However, 3 months after targeted therapy in another hospital, CT revealed tumor enlargement. Although sunitinib, regorafenib and remipatinib were applied in turn, tumor progression appeared. Given the poor efficacy, the patient was admitted to the multidisciplinary team clinic of Zhongshan Hospital for pathology consultation in July 2024. Questioning the original diagnostic results due to the morphology and the clinical response to treatment, a series of immunohistochemical markers were applied to establish the diagnosis. Immunohistochemistry exhibited that LCA and CD3 merely stained lymphocyte, excluding lymphohematopoietic tumors. No CK7, CK8, EMA, CgA, Syn, CD56, WT-1, or Calretinin immunoreactivity was seen in tumor cells, which largely ruled out the possibility of epithelial tumor, neuroendocrine neoplasm and mesothelioma (Fig. 3). OCT4, SALL4, PLAP, SOX17 and D2-40 were strongly expressed in tumor cells, suggesting a possible origin of tumor in the reproductive system (Fig. 4). Different from original unit testing results, sanger sequencing and NGS analysis in Zhongshan Hospital (Shanghai, China) simultaneously detected the point mutation at codon 816 of exon 17 of KIT (p.D816Y) (Fig. 5). NGS workflow was as follows: Nucleic

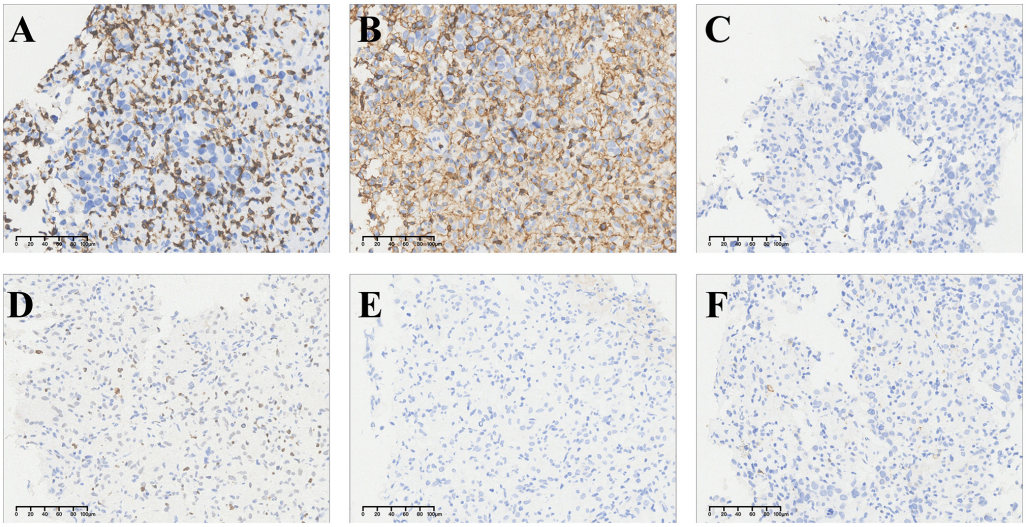

Figure 3. Immunohistochemistry for (A) LCA (positive in lymphocytes), (B) CD3 (positive in lymphocytes), (C) CK7 (negative), (D) CgA (negative), (E) Calretinin (negative) and (F) D2-40 (positive).

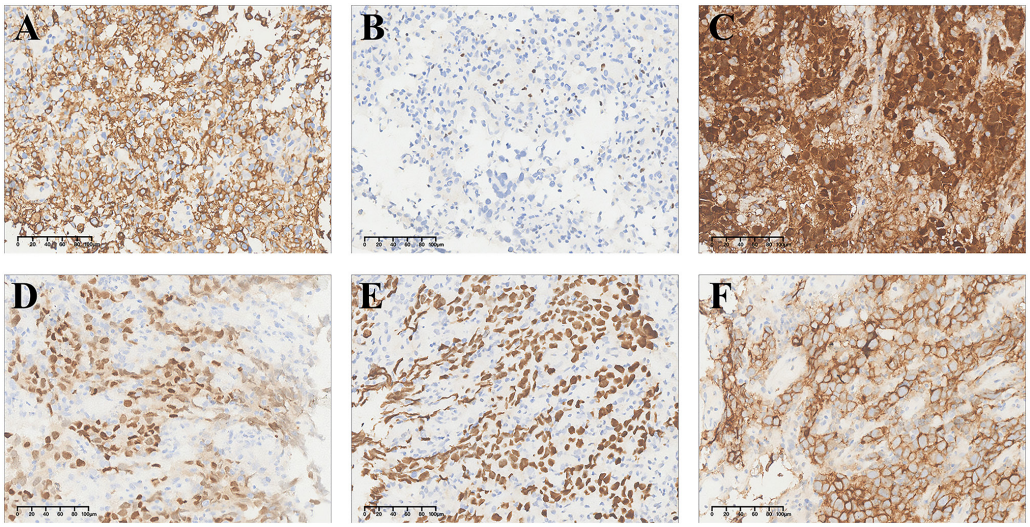

Figure 4. Immunohistochemistry for (A) CD163 (positive), (B) p63 (negative), (C) SOX17 (positive), (D) OCT4 (negative), (E) SALL4 (positive) and (F) PLAP (positive).

acids were extracted from formalin-fixed paraffin-embedded (FFPE) tissue sections using the FFPE DNA Automated Nucleic Acid Extraction Kit (cat. no. 8.02.0071), according to the manufacturer's instructions. DNA concentration was measured by Qubit fluorometer (Thermo Fisher Scientific, Inc.), and the total amount of DNA exacted from tissue samples should exceed 60 ng. The extracted DNA was sheared into fragments of 200-250 bp using a Covaris LE220 system. (Index NGS libraries were constructed through sequential steps of end repair, A-tailing, adaptor ligation, and PCR amplification using the NEBNext® Ultra™ II DNA Prep Kit (cat. no. E7645; NEB). Targeted capture was performed using the AmoyDx® Master Panel covering 571 genes associated with DNA mutations. The resulting libraries were quantified by the Quantus™ Fluorometer, showing a concentration of at least 4.5 pM, and library fragment size distribution was assessed with the Agilent 2100 Bioanalyzer. Pooled libraries were sequenced on an Illumina NovaSeq 6000 platform with 2x150 bp pair-end reads, employing the Illumina Novaseq 6000 S1 Reagent kit v1.5 (300 cycles; cat. no. 20028317; Illumina, Inc.). Demultiplexing and FASTQ file generation were carried out using bcl2fastq v2.17 software (Illumina, Inc.), which also initiated the automated downstream analysis pipeline. Ultimately, a diagnosis of seminoma was confirmed. The present study was performed in accordance with the research policies approved by Zhongshan Hospital, Fudan University [approval no. B2020-101(2)R]. Informed consent was waived from the Ethics Committee of Zhongshan Hospital, Fudan University.

Discussion

Patients with seminoma generally present with a palpable mass and may experience pain. Serum levels of alpha-feto-protein (AFP) are elevated, but this is not a specific marker. Patients with syncytiotrophoblasts have increased serum

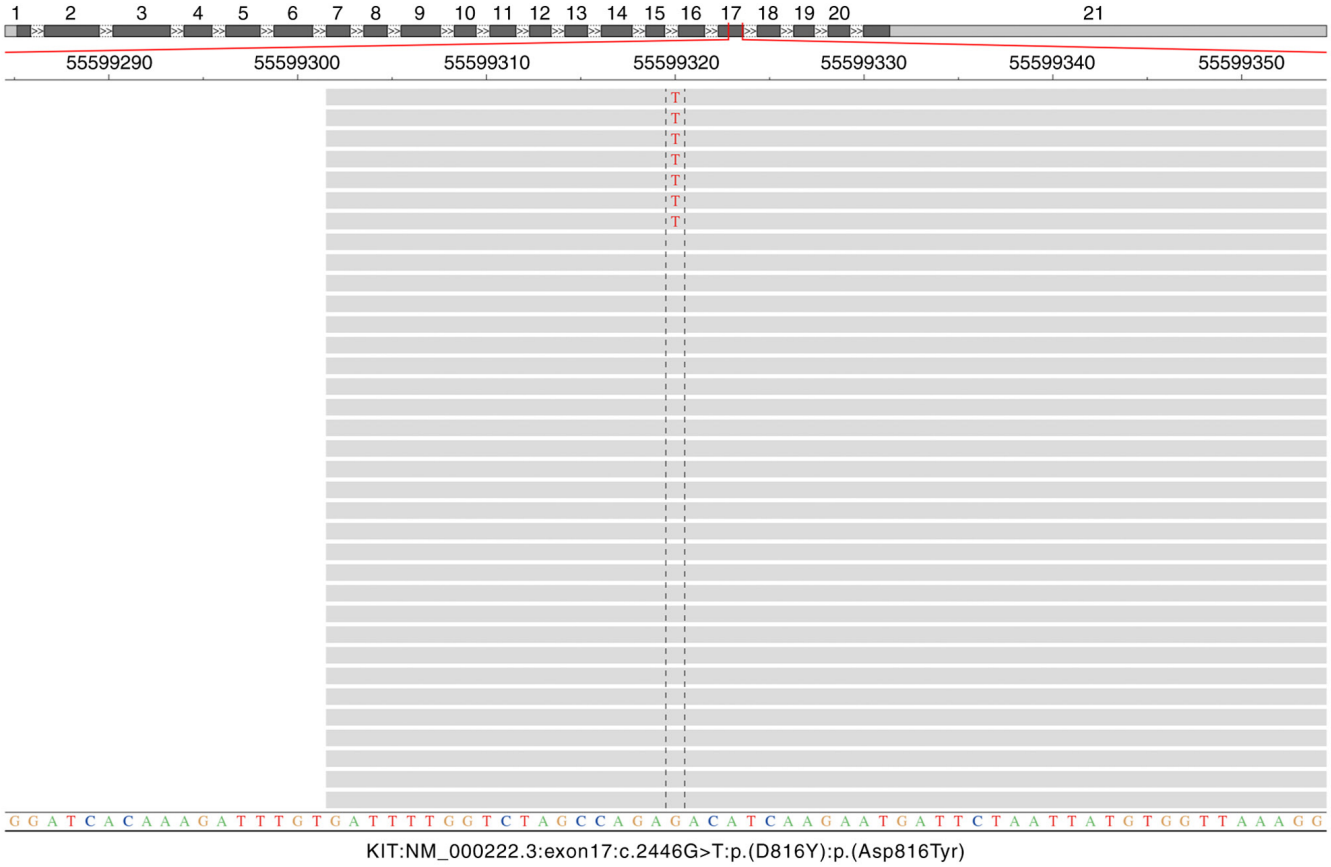

Figure 5. NGS analysis revealed KIT p.816V mutation.

human chorionic gonadotrophin (HCG), usually not exceeding 1,000 mIU/ml. Imaging shows that the mass has well-defined borders and exhibits homogeneous hypoechogenicity.

Gross examination reveals a solid tumor, which is often lobulated, with gray-white to yellow cut surfaces, well-defined margins and homogeneous texture. Hemorrhage or necrosis is barely observed in seminoma; otherwise, seminoma mixed with components from other germ cell tumors may be suggested. Histologically, classical seminoma is characterized by uniformly shaped germ cells arranged in a diffuse sheet-like pattern, which is separated into small lobules, strands, or nests by fibrous vascular septa. Tumor cells have clear boundaries and transparent cytoplasm rich in glycogen. The nuclei are large and centrally located, with regular shapes and clumped chromatin. One or more prominent nucleoli are often visible, along with varying numbers of mitotic figures. Also, the infiltration of different lymphocytes and plasma cells can be observed in the tumor stroma. A total of ~25% of seminomas may show a granulomatous reaction and multinucleated giant cells, which can obscure their original characteristics. Anaplastic seminoma is noted for significant cellular atypia, accompanying with an increased number of mitotic figures (>3/HPF) and few interstitial lymphocytes. Immunohistochemistry can aid in diagnosis when the morphology is atypical. Tumor cells are positive for germ cell markers such as SALL4, OCT3/4, D2-40, PLAP, CD117, and SOX17, while negative for CK, CD30 and AFP (4). HCG, cytokeratin and pregnancy associated proteins are expressed in syncytiotrophoblasts.

The pathological diagnosis in this case contained two critical errors. First, a seminoma was misdiagnosed as a GIST. Second, the genetic analysis revealed a point mutation at codon 816 (p.D816Y) in exon 17 of the KIT gene, which was discordant with the external laboratory report documenting a mutation at codon 823 (p.Y823D) within the same exon. The misdiagnosis of GIST by the external institution may be attributed to a combination of atypical clinical and histopathological features. The patient's presentation with a large abdominal mass instead of a testicular lesion deviated from the classic seminoma phenotype, while limited tissue sampling via biopsy hindered definitive morphological assessment, with tumor cells lacking hallmark characteristics such as clear cytoplasm and prominent nucleoli. Furthermore, the uncritical acceptance of CD117 immunopositivity and KIT mutation detection as pathognomonic for GIST likely contributed to diagnostic inaccuracy, reflecting insufficient integration of morphological, immunohistochemical and clinical data.

A critical reappraisal reveals diagnostic clues that could have alerted pathologists to the misclassification. Morphologically, GISTs predominantly exhibit spindle cell morphology, with mixed or pure epithelioid subtypes accounting for <50% of cases. Notably, such epithelioid GISTs predominantly arise in the stomach and are typically associated with PDGFRA mutations or SDH deficiency, rendering the coexistence of KIT mutations with epithelioid morphology biologically incongruous (5). Furthermore, the immunohistochemical profile in this case, solitary CD117 expression without DOG1 or CD34 co-expression,

deviates from diagnostic standards. As emphasized in the Clinical Practice Guidelines for GIST Diagnosis, definitive GIST classification mandates rigorous exclusion of other CD117-positive neoplasms. The differential diagnosis should comprehensively encompass both epithelial malignancies (for example, adenoid cystic carcinoma, secretory carcinoma, basal cell adenoma, eccrine spiradenoma, renal oncocytoma, chromophobe renal carcinoma, basal cell adenoma, eccrine spiradenoma, renal oncocytoma, chromophobe renal cell carcinoma, thymic carcinoma, pulmonary small cell carcinoma) and non-epithelial tumors (for example, angiosarcoma, granulocytic sarcoma, mast cell neoplasms, malignant melanoma and seminoma), particularly when confronted with atypical morphological and immunophenotypic features (6). Besides, the molecular findings in this case exhibited biological incongruity with typical GIST profiles. Although KIT mutations constitute the most common genetic alterations in GIST, primary tumors predominantly harbor exon 9 (10~15%) or exon 11 (60~70%) mutations (7). Exon 13 and 17 mutations are exceptionally rare in primary GISTs (1~2%), with exon 17 variants historically containing p.A795P (1 case), p.D816F (1 case), p.D816Y (1 case), pD820A (1 case), p.D820Y (1 case), p.D820V (1 case), p.N822K (15 cases), p.N822Y (1 case) and p.Y823D (1 case) (8-10). It is also demonstrated that exon 17 mutation of KIT is mainly found as a secondary gene mutation in drug-resistance GIST after the failure of targeted therapy, with specific codons including N822K (43.94%), Y823D (22.73%), D816H (15.15%), D820Y (6.06%), C809G (4.55%), N822Y (4.55%) and A829P (3.03%) (11,12). Crucially, the mere presence of KIT mutations cannot confirm GIST diagnosis, as such alternations are documented in 8~15% of other CD117-positive malignancies including angiosarcoma, granulocytic sarcoma, mast cell neoplasms, malignant melanoma and seminoma (13). KIT gene mutations occur in 8.2~25.8% of seminomas, predominantly localized to exon 17 (14,15).

The most frequent hotspot involves codon 816 (p.D816V/H), accounting for approximately 35% of cases, followed by codon 882 (p.N822K) with 22% frequency. Additional exon 17 variants (e.g., p. K642E, p.N655K, p.D820Y, p.Y823C, p.Y823D, p.A829T) have been reported at lower frequencies. Exon 11 mutations (3~6%) predominantly affect codons 557 (p.W557S), 576 (p.L576P), and 578 (p.Y578C) (16). Critically, the p.D816V mutation in this case strongly supports seminoma over GIST, as exon 17 mutations are exceptionally rare (<1%) in primary GISTs while prevalent in seminoma.

Different genotypes of KIT mutations are associated with diversified response to specific TKIs. Imatinib, initially developed as a BCR-ABL tyrosine kinase inhibitor, was subsequently identified to potently inhibit KIT receptor tyrosine kinase activity and to be the first-line therapy for GISTs, particularly in patients harboring KIT exon 11 mutation or specific exon 17 variants (for example, p.N22K and p. Y823D) (7). However, several exon 17 mutants, such as p.D816V/H, are resistant to imatinib treatment (13). In the present study, this further explained the disease progression in the short term after imatinib treatment in this patient.

Patients with stage I or localized disease are treated with radical orchiectomy followed by risk-adapted management: Active surveillance (preferred for low-risk patients) or adjuvant therapy based on established risk factors (tumor size >4 cm, rete testis invasion). Stage IIA/B requires post-orchiectomy treatment with either radiotherapy (20~30 Gy to involved fields) or chemotherapy (BEP x 3 cycles). Advanced/unresectable disease mandates first-line cisplatin-based chemotherapy (BEP x 3-4 cycles), achieving 83% disease-specific survival at 5 years (17). Notably, targeted therapies (imatinib/sunitinib/pazopanib) demonstrate limited efficacy with objective response rate <15%, reflecting intrinsic resistance mechanism in germ cell malignancies (18).

There are several limitations in the present study. First, a critical limitation of this report was the unavailability of primary CT imaging findings essential for diagnosing extragonadal seminoma. During pathological consultation at our institution for diagnostic verification, only textual radiological descriptions from an external facility were provided and original DICOM images were unobtainable. Furthermore, no supplemental CT imaging or therapeutic interventions were pursued at our center, constraining comprehensive clinicopathological correlation. In addition, another limitation was the omission of serum tumor markers (AFP, β-HCG and LDH) during initial diagnosis of the case. Although these biomarkers provide substantial diagnostic utility in seminoma detection, particularly in extragonadal presentations, their measurement was overlooked due to the original misdiagnosis of GIST based solely on CD117 immunopositivity and reported KIT mutation. This precluded timely diagnostic rectification through serological correlation.

In conclusion, this diagnostic pitfall underscores the imperative for morphology-driven, multidisciplinary evaluation when interpreting CD117-positive tumors. Solitary CD117 expression requires systematic exclusion of other CD117-positive malignancies to diagnose GIST. Reliance solely on molecular findings risks misclassification. Although KIT mutations support GIST diagnosis in appropriate contexts, rare mutation loci more strongly align with non-GIST neoplasms. Furthermore, interlaboratory variability in molecular alternation necessitates validation by multiple detection methods including sanger sequencing and NGS, and correlation with histomorphology. Notably, rapid disease progression on multi-line targeted therapy, despite reported KIT mutation, should prompt immediate reassessment of diagnostic validity, including expert histopathology review and expanded molecular profiling.

Acknowledgements

Not applicable.

Funding

The present study was supported by the National Nature Science Foundation of China (grant no. 32571067).

Availability of data and materials

The data generated in the present study may be found in the SRA database under accession number PRJNA1304866 or at the following URL: https://www.ncbi.nlm.nih.gov/sra/PRJNA1304866.

Authors' contributions

XZ, WY and YH contributed to the conception and the design of the study. LR and CX contributed to the acquisition, analysis, and interpretation of the data. XZ and YH contributed to drafting the manuscript and revising the manuscript. All authors read and approved the final version of the manuscript. XZ and WY confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The present study was performed in accordance with the research policies approved by Zhongshan Hospital, Fudan University [approval no. B2020-101(2)R]. Informed consent was waived from the Ethics Committee of Zhongshan Hospital, Fudan University.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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