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ORIGINAL ARTICLE

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In classical Hodgkin lymphoma, the activity of Thymidine kinase-1 is not related to tumor size

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ABSTRACT

Introduction: Classical Hodgkin lymphoma (cHL), a common lymphatic malignancy commonly occurring in people aged between 30 and 40, is known to have serum thymidine kinase 1 (TK1) as a confirmed diagnostic marker. However, the relationship between serum TK1 and tumor volume is controversial.

Objectives: Examination of the relationship between serum TK1 activity and lymph node volume in patients with cHL.

Materials and methods: Twenty-four patients with HL underwent PET/CT examinations, and a control group of 30 healthy subjects was established. PET / CT scans were analyzed to determine the maximum standardized uptake value (SUVmax), tumor size, and volume. Serum TK1 levels were measured using the DiaSorin LIAISON assay.

Results: The findings demonstrated a high serum TK1 concentration in cHL patients and further validated TK1 serum as a diagnostic biomarker. However, no statistically significant association was observed between serum TK1 activity and lymph node size. These results suggest that although serum TK1 is a useful diagnostic marker for cHL, Serum TK1 is unreliable for measuring tumor aggressiveness and malignancy.

Conclusions: This study offers new data on the biology of cHL and highlights the importance of continuing research on alternative biomarkers for disease surveillance and prediction.

Keywords: classical Hodgkin lymphoma; thymidine kinase 1; marker

Introduction

Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL) are the two main categories of lymphatic diseases, distinguished by their clinical characteristics. Notably, HL is characterized by the presence of Reed-Sternberg cells, while these cells, as well as specific staining markers such as Cd15 and Cd30, are absent in NHL [1–3]. NHL encompasses more than 40 subtypes, with indolent follicular lymphoma (FL) and aggressive diffuse large B-cell lymphoma (DLBCL) being the most common forms [4, 5].

HL predominantly affects young and elderly patients and includes two primary forms: classical HL (cHL) and nodular lymphocyte-predominant HL (NLPHL). These forms are defined by distinct morphological and immunophenotypic characteristics of the lymphoma cells [6]. The diagnosis of HL is based on the identification of multinucleated giant Reed-Sternberg cells. Several risk factors contribute to the development of cHL, including age, sex, family history, and exposure to Epstein-Barr virus (EBV) [7]. While the exact role of EBV in cHL remains unclear — since most infected individuals do not develop HL — genetic predisposition and co-infection with HIV are known to increase the disease risk [8–11].

The classification of cHL into nodular sclerosis, mixed cellularity, lymphocyte-depleted, or lymphocyte-rich HL is based on histological and immunohistochemical analysis of lymph node tissue [12]. A variety of biomarkers — including genetic alterations, epigenetic alterations, microRNAs, long non-coding RNAs (lncRNAs), immunophenotypic biomarkers, and tumor microenvironment-related biomarkers — facilitate the diagnosis and prediction of HL [13]. Among the immunophenotypic biomarkers, kinases involved in oncogenic signaling pathways play a crucial role. These include Bruton tyrosine kinase (Btk) [14], IκB kinase [15], and NF-κB-inducing kinase [16]. Additionally, thymidine kinase 1 (TK1), which regulates cell turnover through apoptosis in healthy cells [17], may serve as a viable marker for cHL [18, 19].

Materials and methods

This study received approval from the Bioethics Committee of XX (KB/09/2022). A consent waiver was obtained through the Institutional Review Board (IRB) of XX. The research was conducted at the Institute of Hematology and Transfusion Medicine in Warsaw, Poland, and involved 24 patient samples. The basic characteristics of these patients are presented in Table 1. Additionally, a control group consisting of 30 healthy subjects was established.

The histological diagnosis followed the 2008 World Health Organization classification criteria. An experienced radiologist analyzed computed tomography (CT) data obtained from positron emission tomography (PET)/CT imaging. PET scans using 2-[fluorine-18] fluoro-2-deoxy-D-glucose (FDG) (18F-FDG PET) assessed the clinical stage, response to treatment, and tumor size in patients diagnosed with Hodgkin lymphoma (HL).

Before the examination, patients were instructed to fast for at least six hours, and blood glucose measurements were taken before the administration of 18F-FDG. To ensure proper hydration and bladder emptying before the PET/CT examination, all patients were asked to drink 1.5 liters of water. Notably, blood glucose levels for all patients were maintained below 150 mg/dL (8.33 mmol/L). Following standard CT procedures, patients were positioned supine with their arms raised.

PET/CT examinations were conducted 60 minutes after the intravenous injection of 300 to 370 MBq of 18F-FDG using a Biograph 64 TruePoint PET/CT scanner (Siemens Medical Solutions, Knoxville, TN). Initially, low-dose CT scans were performed with a 64-detector helical CT scanner to correct for attenuation. This was followed by a continuous low-dose CT scan in spiral mode using parameters of 120 kV, 170 mA, 2 mm slice thickness, and a pitch of 0.8. The PET study was then performed immediately after the CT, with the patient remaining in the same position. Each bed position was analyzed for 2 minutes, with a total of six or seven positions, depending on the patient's size. PET image data were reconstructed using a 168 × 168 matrix and applied the ordered subsets expectation maximization algorithm (two iterations, 14 subsets), while also being corrected for attenuation using CT data.

The PET/CT images (which included half-body attenuated and non-attenuated PET, CT, and fused images) were transferred to a multimodal workstation (Syngo (TrueD); Siemens Medical Solutions) for analysis. Tumors were quantitatively evaluated by a nuclear physician, who assessed the maximum standardized uptake value (SUVmax) of all visible lesions. The

SUVmax for 18F-FDG in tumors was calculated by drawing a region of interest (ROI) encompassing the central two-thirds of the adrenal mass while excluding peripheral areas to avoid partial volume effects. The density of the ROI was expressed in Hounsfield units. Additionally, the maximum diameter and mean unenhanced attenuation for each visible lesion were calculated, with the total volume of the nodes expressed in cubic centimeters.

Serum thymidine kinase 1 (TK1) levels in patients with classic Hodgkin lymphoma were measured using the DiaSorin LIAISON assay on the LIAISON analyzer. The LIAISON TK assay is an indirect, modified two-step chemiluminescent immunoassay that quantifies thymidine kinase levels in human serum and plasma EDTA. The initial enzymatic reaction involves TK reacting with 3’-azido-3’-deoxythymidine (AZT) to form 3'-azido-3'-deoxythymidine monophosphate (AZTMP). This is followed by an immunoassay that allows for the quantification of AZTMP. The amount of AZT converted to AZTMP reflects the level of thymidine kinase present in the sample.

All statistical analyses were conducted using the R package. Differences between groups were evaluated with the Wilcoxon test, while linear regression was employed to assess the correlation between tumor size and serum TK1 levels.

Results

In healthy subjects, the median serum concentration of TK1 was 4.3 U/L, with an interquartile range (IQR) of 3.13–5.80. In contrast, patients with Hodgkin lymphoma had a median TK1 concentration of 13.75 U/L, with an IQR of 9.92–21.62. Figure 1 illustrates the TK1 activity box plot and highlights the statistical differences between patients with Hodgkin lymphoma patients and healthy subjects.

Figure 2 presents the cross-correlation analysis between TK1 activity and lymphoma volume. In particular, the present results do not show a significant correlation between serum TK1 levels and lymphoma volume, with a correlation coefficient of $\rho = -0.17$ ($p = 0.43$).

Discussion

To the authors' knowledge, this study is the first to investigate the relationship between TK1 protein activity in classical Hodgkin lymphoma (cHL) serum and lymphoma size. The present findings indicate that serum TK1 activity is higher in cHL patients than in healthy individuals, consistent with a previous study by Ulfstedt et al. [18]. This study also confirms previous reports suggesting that elevated serum levels of TK1 may indicate tumor presence [20–22]. Although TK1 is not a tumor marker per se but rather a cell proliferation marker, its activity is directly related to DNA synthesis and, therefore, should correlate with cancer aggressiveness [23]. In cHL, Reed-Sternberg cells, which are a minority of cells within the malignant lymph node [24], are surrounded by a large number of metabolically active mononuclear cells that contribute to FDG uptake. Logically, uptake by lymph node volume would usually be directly related to increased cell proliferation, which serum TK1 could trace.

Furthermore, serum TK1 has been proposed as a liquid-based proliferation marker for highly aggressive tumors [25, 26]. However, the present investigation revealed a surprising outcome. Contrary to expectations, this report shows that serum TK1 activity does not correlate with cHL node volume and malignancy.

Limitations of the study

One of the limitations of this study is the restricted sample size and the variability in the methods used to measure TK1 activity and lymph node volume, which may compromise the reliability and consistency of the results. Furthermore, the biological mechanisms underlying the relationship between TK1 activity and lymphoma volume are not yet fully understood, which limits the interpretation of the findings.

Conclusions

This investigation examined the correlation between serum thymidine kinase 1 (TK1) activity and lymph node size in individuals diagnosed with classical Hodgkin lymphoma (cHL). The present research reveals that serum TK1 activity is significantly elevated in patients with cHL compared to healthy controls, implying its potential as a diagnostic indicator of tumor presence. However, contrary to the initial hypothesis, no significant correlation was found between serum TK1 levels and lymph node size, suggesting that TK1 activity may not be a

reliable predictor of lymphoma aggressiveness. These findings have significant implications for the diagnosis and management of cHL, emphasizing the need for further exploration into the role of TK1 in this disease. Despite the lack of correlation, serum TK1 remains a promising biomarker for cancer detection and monitoring, which warrants further investigation of its clinical utility.

Article information

Data availability statement: *The data reported in this study are available on request from the authors.*

Ethics statement: *A consent waiver was obtained through the IRB of the University of Technology and Humanities, Radom, Poland.*

Author contributions: *R.T. and L.K. conceived the presented idea and performed experiments. M.W. developed the theory and performed the computations. All authors discussed the results and contributed to the final manuscript.*

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

Supplementary material: *None*.

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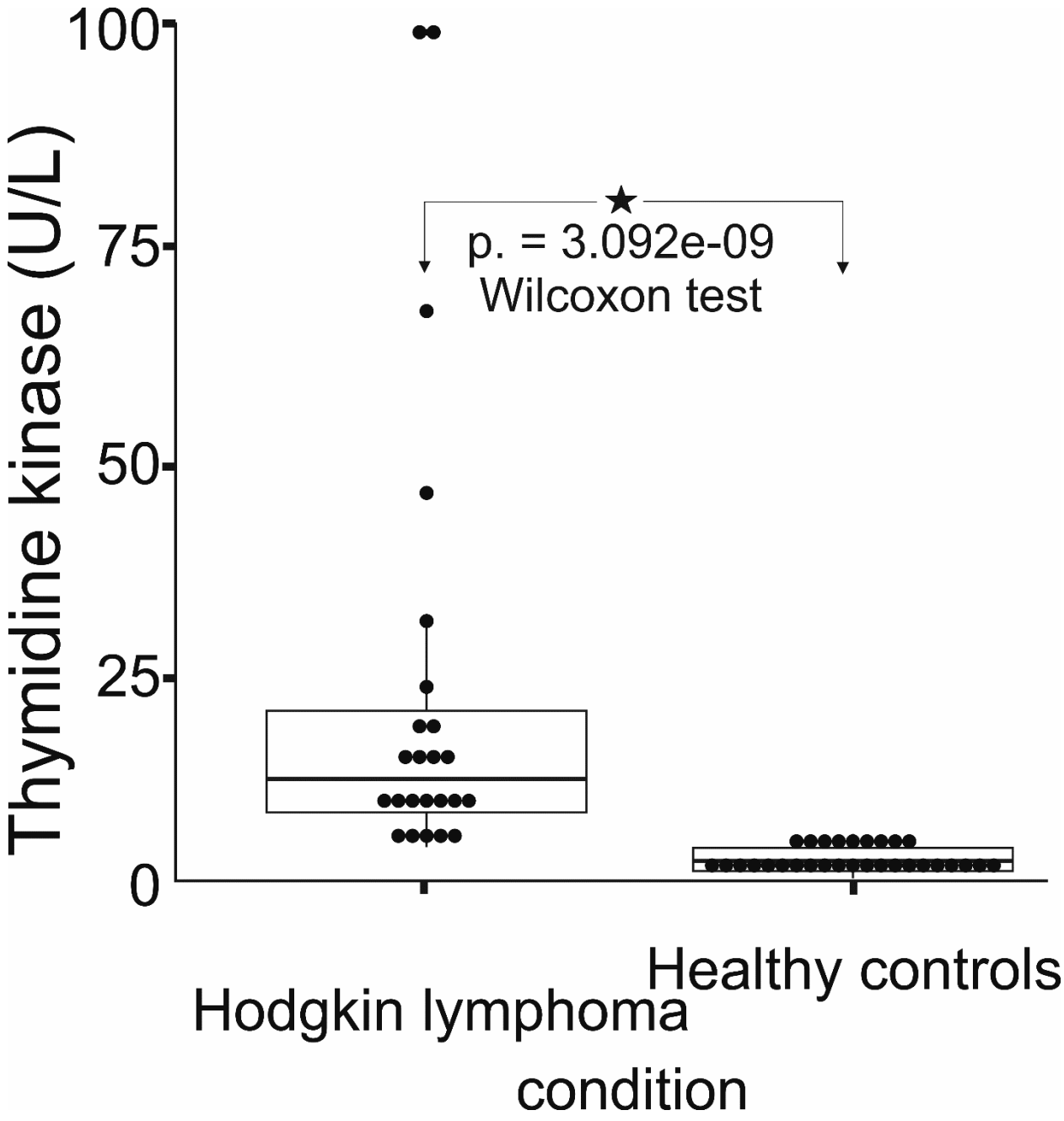

Figure 1. The difference in thymidine kinase activity between healthy control and Hodgkin lymphoma patients

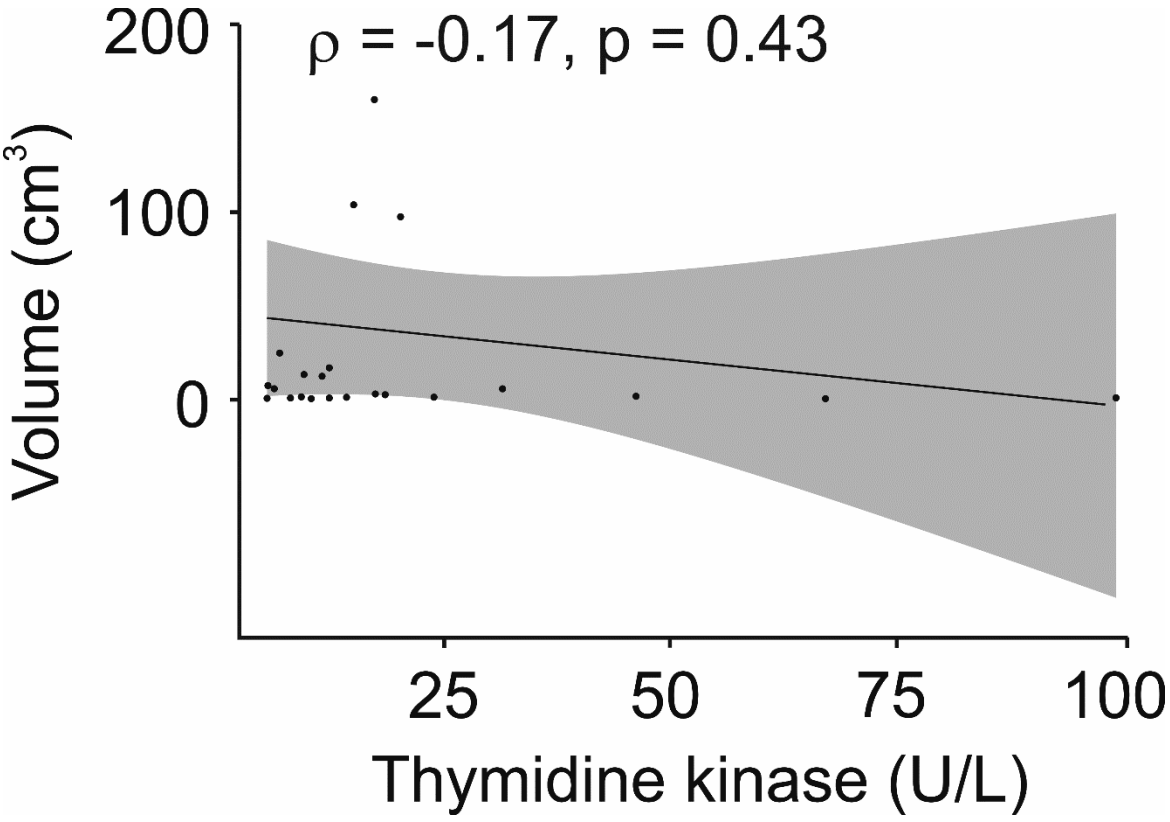

Figure 2. Correlation between Hodgkin lymphoma node volume and thymidine kinase activity

Table 1. Clinical characteristics of studied patients

Variable	**cHL (n = 24)**
Sex	male
Age median (min–max)	47 (18–76)
Histology subtype	Nodular sclerosis
Ann Arbor Stage	I–IV
Epstein–Barr virus (EBV)	Negative
B-Symptoms	present
International Prognostic Score (IPS)	1–3
Chemotherapy	Adriamycin, Bleomycin, Vinblastine, Dacarbazine (ABVD)

cHL — Classical Hodgkin lymphoma