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## 624.HODGKIN LYMPHOMAS AND T/NK CELL LYMPHOMAS: CLINICAL AND EPIDEMIOLOGICAL

**The Lymphocyte Subsets Activation Profiles in Peripheral Blood May Contribute to the Diagnosis of Natural Killer/T-Cell Lymphoma-Associated Hemophagocytic Lymphohistiocytosis**

Yanxia He [1], Yan Gao [1], Xiaoxiao Wang [1], Bing Bai [1], Zhiming Li [1], Haixia He [2], Liqin Ping [1], Cheng Huang [1], Haoqing Chen [1], Huiqiang Huang, MDPhD [3]

[1] Department of Medical Oncology, Sun Yat-sen University Cancer Center, State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, Guangzhou, China
[2] Department of Radiation Oncology, Sun Yat-Sen Memorial Hospital, Guangzhou, China
[3] Department of Medical Oncology, Sun Yat-Sen Univ. Cancer Ctr., Guangzhou, China

**The lymphocyte subsets activation profiles in peripheral blood may contribute to the diagnosis of natural killer/T-cell lymphoma-associated hemophagocytic lymphohistiocytosis**

Background: Hemophagocytic lymphohistiocytosis (HLH) is a rampant and potentially fatal systemic hyperinflammation characterized by dysfunction of macrophages and cytotoxic T cells. Natural killer/T-cell lymphoma (NKTCL) has been reported to be one of the most common etiologies of HLH among hematologic neoplasms. Although, the diagnosis of natural killer/T-cell lymphoma-associated hemophagocytic lymphohistiocytosis (NK/T-LAHS) refers to the HLH-2004 diagnostic guidelines for primary HLH, most of them are based on non-specific clinical features. Identification of the immunophenotype characteristics is important for the diagnosis of NK/T-LAHS. In this study, we investigated the lymphocyte subsets activation profiles of peripheral blood mononuclear cells (PBMC) from NKTCL patients, and preliminarily screened molecular marker with potential diagnostic value for NK/T-LAHS.

Methods: This study retrospectively collected peripheral blood samples from 58 patients diagnosed with NKTCL in Sun Yat-sen University Cancer Center from September 2020 to December 2022. Among them, 29 patients had HLH at the time of enrollment, and peripheral blood samples from 10 healthy people were collected as the control group. NKTCL was diagnosed according to the 2016 WHO classification of malignant lymphoma. All HLH patients met HLH-2004 diagnostic criteria and primary HLH was excluded. Flow cytometric staining of PBMC was performed on BD Fortessa according to the manufacturer's instructions. All samples were analyzed by Flowjo(V10)software. The ROC curve was drawn according to the percentage of lymphocyte subsets to determine the optimal cutoff threshold. RStudio 4.2.1 was used to calculate the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and likelihood ratio (LR) of the related indicators. Graphpad prism 8.0.1 and SPSS 26.0 (IBM) was used for drawing and statistical analysis. All statistical tests were two-sided, and $p<0.05$ were considered statistically significant.

Results:The median age of 58 patients was 41 (range, 18-83) years old, and 10 patients (17.2%) had poor performance status (ECOG PS≥2). Forty-two patients (72.4%) were stage III-IV, and 26 patients (44.8%) had PINK-E score≥3. Firstly, we compared the immune cell activation profiles between healthy donors, NKTCL patients with or without HLH. We observed that the proportion of functionally activated T cells, NK cells, NKT cells that expressed CD86, CD69, or HLADR was significantly higher than healthy donors ( $p<0.05$ ), except for CD86+NK cell levels was no significance between the three groups (Figure1). In addition, the percentage of CD86+NK/T cells, CD69+NK cells, HLADR+NK cells and HLADR+NKT cells were significantly higher than NKTCL patients without HLH and healthy controls( $p<0.05$ ), and no significant difference of these immune cells subsets between those non-HLH patients and healthy controls was observed (Figure1). Based on the significance of these lymphocyte subsets in NKTCL patients with and without HLH, we evaluated the diagnostic value of CD86+NKT cells, CD69+NK cells, HLADR+NK cells, and HLADR+NKT cells in NK/T-LAHS patients by ROC analysis to determine the optimal diagnostic threshold. We observed that HLADR+NK > 15.9% could better distinguish NK/T-LAHS from non-HLH patients, with a sensitivity of 0.759 (95%CI: 0.603-0.914) and specificity of 0.828(95%CI:0.690-0.965), and the AUC was 0.775 (95%CI:0.647-0.904) (Table 1). HLADR+NK cells may have potential application value for the diagnosis of NK/T-LAHS.

Conclusions: These activated T cells, NK cells, and NKT cells that express functional activation markers was increased in NK/T-LAHS patients. Among them, HLADR+NK > 15.9% could better distinguish NK/T-LAHS from NKTCL patients without HLH

and healthy donors, which may be a potential biomarker for the diagnosis of NK/T-LAHS patients and contribute to better management.

**Disclosures** No relevant conflicts of interest to declare.



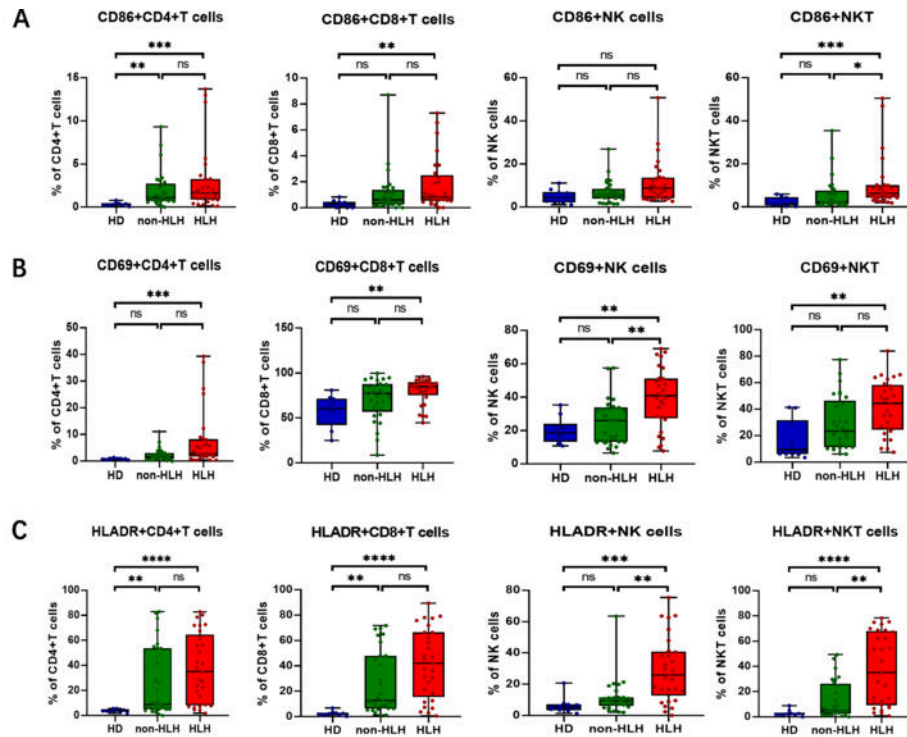**Figure 1. Comparison of immune cell activation profiles between health Donors (n=10), NKTCL patients with HLH (n=29) and NKTCL patients without HLH (n=29).** Boxplots show the median and the range of maximum and minimum values for each lymphocyte subset. The Kruskal-Wallis test was used to compare the differences between groups. *$p<0.05$, ** $p< 0.01$, *** $p<; 0.001$, **** $p< 0.0001$, ns: no significance, HD, Health Donors.

**Table 1. NK cell and NKT cell activation markers that distinguish NK/T-LAHS from non-HLH patients**

| Activation marker | Sensitivity | Specificity | PPV | NPV | LR | AUC |
|---|---|---|---|---|---|---|
| CD69+NK >36.0% | 0.655 (0.482-0.828) | 0.862 (0.737-0.988) | 0.826 (0.671-0.981) | 0.714 (0.565-0.864) | 4.75 | 0.753 (0.624-0.883) |
| HLADR+NK >15.9% | 0.759 (0.603-0.914) | 0.828 (0.690-0.965) | 0.815 (0.668-0.961) | 0.774 (0.627-0.921) | 4.4 | 0.775 (0.647-0.904) |
| HLADR+NKT >51.4% | 0.448 (0.267-0.629) | 1 | 1 | 0.644 (0.505-0.784) | Inf | — |
| CD86+NKT >2.8% | 0.897 (0.786-1) | 0.586 (0.407-0.765) | 0.684 (0.536-0.832) | 0.85 (0.694-1.006) | 2.167 | — |

**Abbreviation:** PPV, positive predictive value; NPV, negative predictive value; LR, likelihood ratio.

**Figure 1**