NODAL T-FOLLICULAR HELPER CELL LYMPHOMAS AND PERIPHERAL T-CELL LYMPHOMAS, NOT OTHERWISE SPECIFIED IN SLOVENIAN PATIENTS: MUTATIONAL LANDSCAPE, CLINICOPATHOLOGICAL CHARACTERISTICS AND OUTCOMES

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2

Abstract

Background: Nodal T-follicular helper cell lymphomas (nTFHLs) are the most frequent mature nodal peripheral T-cell lymphomas (nPTCL). Understanding of mutational landscape and its correlation with survival outcomes remains limited. We aimed to better depict nPTCLs' features within a Slovenian cohort.

Methods: 108 patients diagnosed with nPTCL were studied; high-throughput sequencing with a lymphoma panel of 172 genes was performed. Survival analyses with clinical and mutational characteristics and correlation analyses were done

Results: Out of 108 patients (50 females, 58 males), 91 had nTFHL, 9 PTCL, not otherwise specified (PTCL-NOS) and 8 composite lymphomas [CLs; co-occurrence of nTFHL, angioimmunoblastic type (nTFHL-AI) and monoclonal B-cell proliferations]. The median follow-up time was 23 months (range 0-156). Most patients received COP/modified COP treatment. Mutations of *TET2* was present in 43%, *RHOA* in 26%, *IDH2* in 9%, *PLCG1* in 8%, *DNMT3A* in 6%, and *VPS13B* in 4% of the cases, in nTFHL only. Correlation analysis revealed an association between mutations in RHOA and the presence of mutations in *TET2, IDH2,* and *DNMT3A*. No statistically significant association was found between the main clinical characteristics and *TET2* or *RHOA* mutations. Patients with high International prognostic index (IPI), progressive disease after the first-line treatment, without autologous stem cell transplantation (ASCT) as consolidation therapy and ≥2 mutations had shorter overall survival (OS), all being independent adverse risk factors.

Conclusion: A variety of recurrent mutations were observed in our nPTCL cohort; simultaneous presence of ≥2 mutations portend a higher risk of death. Our results emphasize the importance of ASCT as consolidation therapy and need for prospective clinical trials for tailored therapeutic interventions like application of hypomethylating agents in *DNMT3A* and/or *TET2* mutated instances or IDH-inhibitors in *IDH2* mutated ones to improve patient outcomes.

Nodal peripheral T-cell lymphomas (nPTCLs) are rare and heterogeneous diseases with poor prognosis (1). Understanding of their pathogenesis have improved due to the recent advancements in mutational profiling research (1,2). Similar mutations can occur across different nPTCL subtypes but their distribution vary (2-4). Nodal T-follicular helper cell lymphomas (nTFHL), frequently exhibit mutations in epigenetic regulators (*TET2, DNMT3A, IDH2*) as well as mutations that lead to T-cell activation, such as *RHOA* (2-4). These mutations are present in other PTCL subtypes but at lower frequencies. Other less frequent mutations, present in several PTCLs, occur in co-stimulatory or TCR signalling genes such as *CD28, PLCG1, CARD11*, and *CTNNB1* (2-4).

Mutations in epigenetic regulators in nTFHLs have significant clinical implications as well. Respectively mutated lymphomas respond better to histone deacetylase (HDAC) and DNA

methyltransferase inhibitors in comparison to non-mutated one. Some of the gene mutations or the presence of co-mutations affect survival outcomes in PTCLs. However, the prognostic impact of these mutations remains largely unexplored. In PTCL-NOS, alterations in *TP53, PRDM1, PTEN, CDKN2A/B, STAT3*, and *MYC* are linked to the more aggressive PTCL-GATA3 subtype (5). In contrast, PTCL-TBX21, which harbours mutations in *TET1, TET3*, and *DNMT3A*, has a better prognosis (5).

There is limited information on the incidence, prognostic markers, mutational landscape, and survival outcomes of nTFHLs and PTCL-NOS in South-Eastern Europe. Our study aims to characterize molecular and clinical aspects in Slovenian patients diagnosed with nTFHL and PTCL-NOS.

Hundred-eight Slovenian patients diagnosed with nTFHL, PTCL-NOS, and composite lymphomas (CL, i.e. co-occurrence of nTFHL-AI and B-cell lymphoma) and who underwent treatment at the Institute of Oncology Ljubljana (IOL), Slovenia between 2007 and 2022 were included in the study. Clinical and laboratory data were obtained from the patients' electronic medical records in the hospital information system.

DNA was isolated from 10μmthick sliced untreated FFPE tissue samples by using the Invitrogen™ RecoverAl™ Multi-Sample RNA/DNA Workflow (#A26069 Thermo Fisher Scientific, Waltham, MA, USA). Only cases with a tumor cell content (TCC) >15% were deemed eligible for next-generation sequencing (NGS). NGS was performed using IonAmpliSeq™ customized, validated and ISO15189 accredited lymphoma panel comprising 4716 amplicons (size range: 125 – 175) of 172 genes (Thermo Fisher Scientific, Waltham, MA, USA).

Descriptive statistics summarized the clinical data. Statistical analyses were conducted using SPSS software (version 28.0.1.0, IBM, Armonk, NY, USA) and RStudio. The R package "BiocManager (maftools)" was used to create an oncoplot with a mutational hierarchy. Pearson Chi-square or Fisher's exact test were used for the correlation analyses of categorical variables, the Kaplan-Meier method (with log-rank test) to generate survival curves and to evaluate differences in overall survival (OS). OS was defined as the time from pathological diagnosis to death from any cause or end of follow-up. The Cox proportional hazards model was applied to assess the independent impact of potential prognostic factors on survival outcomes. Variables with $p \leq 0.10$ in the univariate analyses were included in the multivariate analysis. We excluded potential confounding factors that were already part of other variables, such as ECOG PS and LDH, which are components of the IPI score. Differences with $p < 0.05$ were considered statistically significant. Patient vital status was retrieved from the Cancer Registry of t Study cohort was comprised of 58 males (54%) and 50 females (46%), with a median age of 69 years and a range of 26–87 years. Elevated serum LDH levels were detected in 69 patients (64%), and 68 patients (72%) presented with B-symptoms. Three-quarters of patients (n=75; 69%) had good ECOG PS (0–1) at diagnosis. Most patients (n=100; 93%) were diagnosed at advanced stages (Ann Arbor stage III-IV), and 63% were in the IPI high-intermediate or high-risk group. Median patient survival was expressed in months.

Eight patients underwent autologous stem cell transplantation (ASCT) as consolidation therapy after initial treatment. No significant difference (p=0.522) in OS was observed between patients receiving different first-line treatments. However, a significant difference (p<0.001) was detected when comparing patients with different response rates to first-line treatment. Patients with ASCT consolidation therapy experienced prolonged OS compared to those who did not (p=0.006). The multivariate Cox proportional hazards model revealed that ASCT as consolidation therapy was a favourable prognostic factor. In contrast, disease progression after first-line treatment was an adverse independent prognostic factor. Patients with progressive disease (PD) after first-line treatment had a 9.878 (95% CI 4.018-24.289, p=<0.001) times higher risk of death compared to patients with a complete response (CR) (Table 3). There was also a trend toward worse prognosis in patients with PR compared to those with CR (HR 2.024, 95% CI 0.935-4.425, p=0.079).

Ninety-nine samples were sequenced, however, 9 were excluded due to DNA fragmentation. Among

the 90 patients with interpretable results, 22 had no detected clinically significant mutations, 24 had 1 mutation, and 53 had ≥2 mutations in different genes. Mutations in the *TET2* gene were the most common (n=43, 43%), followed by *RHOA* (n=26, 26%), with the *RHOAG17V* variant representing 88.5 % of these mutations (n=23/26). *IDH2* was mutated in 9%, *PLCG1* in 8%, *DNMT3A* in 6%, and *VPS13B* in 4% of patient samples. All these mutations were found exclusively in nTFHLs. Otherwise, heterogeneous mutations were present. Among them, only the *IDH2* mutation was subtype-specific, occurring solely in nTFHL-AI patients (12.3%).

We confirmed mutations in *TET2* and *RHOA* in nTFHL-AI in higher percentages (50% and 34%, respectively) than in other nTFHL subtypes (24% and 18%), while *PLCG1* (9% vs. 12%) and *DNMT3A* (4% *vs.* 18%) in lower percentages. In CL the percentage of *TET2* (63%) mutations was higher, with no detected mutations in *RHOA* and *DNMT3A*, whereas in *PTCL-NOS* (n=6), mutations in any of these genes were not detected.

There was a significant association between the presence of mutations in *RHOA* and *IDH2* (χ^2=27.796, p= <0.001), *TET2* (χ^2=6.915, p=0.009), and *DNMT3A* (χ^2=10.742, p=0.004). In 9 patient samples, *RHOA* mutations co-occurred with *IDH2*, in 17 with *TET2*, and in 5 samples with *DNMT3A*. All detected *IDH2* mutations (n=9) were the *IDH2R172* variant. In all 9 cases, $IDH2^{R172X}$ co-occurred with $RHOA^{G17V}$ mutations, and in 7 (78%) cases with *TET2* mutations. Altogether we identified 169 different mutations in all analysed genes included in the panel. Most of them were missense mutations (48%), followed by nonsense mutations (28%) and frameshift-INDELs (22%). The remaining 2% were non-frameshift deletions or splice site mutations. All mutations in the *RHOA, IDH2, DNMT3A,* and *PLCG1* genes were missense mutations. In contrast, *TET2* mutations included various types: nonsense (43%), frameshift-INDELs (39%), missense (15%), and others (3%).

At the end of the follow-up period, 81 out of 108 patients died (75%), 69 due to lymphoma. The median follow-up time was 23 months (range 0–156 months). Univariate analysis showed a statistically significant association between IPI risk groups, response rate to the first treatment and ASCT and OS. In contrast, no significant association between individual most frequent mutations of our cohort (T*ET2, RHOA* and *IDH2*) and the OS was observed. Patients with *TET2/RHOA* or *TET2/IDH2* co-mutations showed no difference (p=0.739 and p=0.811, respectively) in OS compared to those with *TET2* mutations alone. We conducted additional analysis to examine how the number of mutations affects OS, revealing a significant difference (p=0.01) in OS across groups with cummlativley ≥2 mutations of diffent genes. Multivariate analysis revealed that a high IPI score (4 and 5) (p=0.002), presence of 1 mutation (p=0.042), but especially presence of ≥2 mutations (p=0.006) were identified as independent adverse risk factors. Patients in the high-risk IPI group had a 5.319-fold higher risk of death (95% CI 1.823-15.521) compared to those in the low-risk group. Additionally, patients with 1 mutation or ≥2 mutations also faced a higher risk of death (HR 2.869, 95% CI 1.041-7.906 or HR 3.334, 95% CI 1.417-7.848) compared to those without mutations.

This study investigated the mutational landscape of nPTCL in a Slovenian cohort, providing additional data on mutations previously reported (2-5), and emphasis on the prognostic value of frequently mutated genes in these patients. Our results align with those of previous studies confirming a similar mutation profile for nTFHL-AI and other nodal lymphomas of TFH-cell origin. Differences in OS among patients were observed based on the number of mutations, response rates to first-line treatment, ASCT consolidation, LDH levels, and stratification into different ECOG PS or IPI risk groups. For the first time, we reported that nPTCL patients with particularly ≥2 mutations in different genes have a higher risk of death compared to those without mutations. This finding establishes the number of mutations as an independent adverse prognostic factor alongside a high IPI score, progressive disease after first-line treatment, or the absence of ASCT as consolidation treatment.

We observed frequent mutations in *TET2* (43%), *RHOA* (26%), *IDH2* (9%), *PLCG1* (8%), *DNMT3A* (6%), and *VPS13B* (4%) (Figure 2), being present in nTFHL only, and discovered that

TET2 gene was in 42 % affected by more than one mutation, consistent with numerous publications. Correlation analysis showed a significant association between mutations of the *RHOA* gene with mutations in *IDH2* ($p < 0.001$), *DNMT3A* ($p = 0.004$), and *TET2* ($p = 0.009$), as demonstrated in previous publications. We confirmed that *TET2, RHOA*, and *DNMT3A* mutations are more frequent in nTFHL-AI (50%, 34%, and 4%) compared to other nTFHL subtypes (24%, 18%, and 18%). In contrast, the proportion of *TET2* mutations is higher in CL (63%), while no mutations in these genes were detected in the six cases of PTCL-NOS.

The observed mutational profile in Slovenian patients appears to align closely with those reported in patient cohorts from Western Europe and Asia (2-6). This similarity suggests universal genetic nTFHL lymphomagenesis gate-keeping mechanisms that are rather tumor-specific than population specific.

The adverse impact of *TET2, RHOA,* and *DNMT3A* mutations on survival outcome in nTFHL has been documented in several studies (2-4). These studies observed shorter progression-free survival (PFS) in patients carrying mutations in these genes, but no effect of individual mutations on OS. Consistent with these findings, our study did not find any significant association between the most frequently observed individual mutations (*TET2, RHOA*, and *IDH2*) and OS.

Consistent with previous reports (2-4) a relatively high percentage of nTFHL patients in our study also presented without mutations (n=22/99; 22.2%). This led us to investigate whether the accumulation of mutations affects OS. We found a significant difference in OS between groups of nPTCL patients with varying numbers of mutations (Figure 4). Multivariate analysis confirmed that patients with one mutation had a marginally significant 2.869-fold higher risk of death (95% CI 1.041-7.906, p=0.042), while those with two or more mutations had a strongly significant 3.334-fold higher risk of death (95% CI 1.417-7.848, p=0.006) compared to patients without mutations. Thus, our study promotes the number of mutations as an independent adverse prognostic factor for OS in nPTCL, and especially nTFHL. To our knowledge, no other studies have noted this association.

The present study for the first time suggests that the accumulation of mutations in nPTCL negatively impacts OS, with the number of mutations serving as an independent prognostic factor alongside the IPI, response to first-line treatment, and ASCT consolidation therapy. The mutational landscape in Slovenian patients' mirrors those observed in previous studies, with frequent mutations in *TET2, RHOA, IDH2, DNMT3A,* and *PLCG1* genes, and notable co-occurrence among some of them. Additionally, our results highlight the importance of genetic profiling in routine diagnostics, prognostication, and possibly for tailored treatment strategies in nPTCL.

References

1. WHO classification of the tumors of hematopoetic and lymphoid tissue 5th Ed, 2022.
2. Feldman et al; Virch Arch 2023.
3. Chen HZ et al; Curr Med Sci 2022.
4. García-Sancho AM et al; Br J Hemat 2023.
5. Amador C et al; Blood 2019.
6. Fukumoto K et al; Cancer Sci. 2018.