Clinico-pathologic Profile of Filipino Patients Diagnosed with Diffuse Large B-Cell Lymphoma, Germinal Center or Non-germinal Center Subtype Treated in a Public Tertiary Hospital from 2016 to 2021

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ABSTRACT

Background. Diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin lymphoma (NHL). Classification of DLBCL is often based on the cell of origin (COO), distinguishing between germinal center B-cell (GCB) and non-GCB subtypes. Although not yet recognized as a distinct entity by the World Health Organization (WHO), double expressor lymphoma (DEL), characterized by the co-expression of c-MYC and BCL2, carries an unfavorable prognosis for a subgroup of DLBCL patients. Another entity is the so-called high-grade B-cell lymphoma with MYC and BCL2 and/or BCL6 rearrangements (double-hit/triple-hit lymphomas) diagnosed through fluorescent in-situ hybridization (FISH) analysis.

Objective. This study aimed to determine the clinicopathologic profile and survival outcomes of Filipino DLBCL patients at the Philippine General Hospital (2016-2021), comparing double-hit versus non-double-hit and double-expressor versus non-double-expressor lymphomas, and assessing concordance between FISH-measured double-hit and IHC-measured double-expressor statuses.

Methods. This is a single-arm, retrospective cohort study involving all surgical pathology cases signed out, with the aid of immunohistochemistry (IHC) studies, as NHL DLBCL, GCB, or non-GCB subtype, from 2016 to 2021. A second panel of IHC studies and FISH analysis using tissue microarray was subsequently done. Most cases exhibited a non-GCB subtype and were classified as DEL on second IHC panel. Five out of eleven DEL cases were reclassified as double hit lymphoma (DHL).

Results. Clinically, most patients with these lymphomas present at age 60 years and below, exhibit B symptoms, with elevated serum lactate dehydrogenase (LDH) levels, at least stage III-IV disease at diagnosis, and possess a high International Prognostic Index (IPI) score, collectively indicating a poor prognosis.

Conclusion. Survival outcomes for patients with DLBCL ranges from three to 37 months. All cases of mortality were associated with DEL, contrasting with DHL cases which had variable outcomes. Due to limited sampling, statistical significance of the results cannot be determined. A comprehensive evaluation is essential to the diagnosis of DLBCL and DHL to include a complete immunohistochemistry panel and molecular testing, notably with FISH studies.

Keywords: lymphoma, diffuse large B-cell lymphoma, cytogenetics, immunohistochemistry

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INTRODUCTION

Lymphoma refers to a malignancy arising from lymphocytes and their precursor cells, typically manifesting as a distinct mass.[1] Broadly, lymphomas are classified into Hodgkin lymphoma and non-Hodgkin lymphoma (NHL). Among NHL, subcategories are defined based on morphology, immunophenotype, genotype, and clinical features.

Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of NHL that is composed of B-lineage large lymphoid cells. Foucar et al. characterized DLBCL as B-cells with nuclear sizes equal to or greater than those of normal macrophage nuclei, or more than twice the size of normal lymphocyte nuclei, exhibiting a diffuse growth pattern.[2] The World Health Organization (WHO) further categorized DLBCL into morphological variants, molecular subtypes, and distinct disease entities.[3] However, a significant proportion of cases displays biological diversity that defies classification based on the aforementioned criteria. As a result, such cases do not necessitate further subclassification and are labeled as DLBCL, not otherwise specified (NOS).

Prognosis of patients with DLBCL involves an interplay of several factors which includes age, International Prognostic Index (IPI) score, molecular cell of origin (COO) subtype, and the presence/absence of specific chromosomal rearrangements or protein expression.[4] The conventional treatment for DLBCL over the years has been the R-CHOP protocol (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone), although outcomes have shown variability.

Gene expression profiling (GEP) is considered the gold standard in subclassifying DLBCL into germinal center B-cell subtype (GCB) and activated B-cell subtype (ABC). A study by Lenz et al. have found that patients with a GCB subtype that was GEP-defined had a more favorable 3-year progression-free survival (PFS) compared with an ABC subtype (74% vs. 40%).[5] Even with the advancements in modern technology, laboratories particularly in developing countries, lack access to the aforementioned test. As a result, algorithms using immunohistochemistry studies have become a surrogate method for estimating the COO in DLBCL. Most of these algorithms have been successful in identifying the COO of GCB DLBCL. However, they lack specificity for the ABC subtype. This finding has led to an important distinction in the nomenclature of DLBCL. The COO of DLBCL as defined by GEP should be classified as GCB or ABC, whereas IHC-defined COO should be classified as GCB or non-GCB. This disparity between the GEP-defined and IHC-defined COO has led some experts to the realization that IHC algorithms cannot precisely reproduce transcriptional signatures. Despite this observation, IHC continues to be extensively utilized in laboratories globally, especially in resource-poor nations as a surrogate test for GEP. The Hans algorithm is the most widely used and the simplest among the algorithms because it utilizes three relatively common and readily available immunohistochemical stains specifically CD10, BCL6, and MUM1. The concordance rate with GEP using this algorithm is at 80%.[6-8]

A study by Rosenwald et al. reported that majority of patients with non-GCB are 60 years old and above. Many studies have also confirmed that patients with the non-GCB COO have a poorer prognosis compared to those with GCB COO.[9]

In the fourth edition of the WHO classification, a subgroup of lymphomas previously identified as B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma has undergone redefinition. It has now been reclassified as high-grade B-cell lymphoma with MYC and BCL2 and/or BCL6 rearrangements. When MYC and BCL2 or BCL6 are implicated, the condition is termed DHL, and if all three genes are involved, it is labeled as triple-hit lymphomas (THL). The detection of these rearrangements in these lymphomas is accomplished through cytogenetic/molecular methods such as fluorescent in-situ hybridization (FISH). Numerous studies have indicated that patients with DHL or THL exhibit an unfavorable prognosis and do not respond well to standard therapy.

Another distinct entity is the so-called DEL. This is identified through IHC studies that reveal the co-expression of both MYC and BCL2. While not yet officially acknowledged by the WHO, DEL is associated with a poor prognosis in patients. A study by Teoh et al. in 2017 established those individuals with the non-GCB subtype, coupled with the co-expression of MYC and BCL2, constitute a distinct group with a markedly unfavorable prognosis, regardless of ethnic background.[10]

While both DHL and DEL are associated with an unfavorable prognosis, using co-expression in IHC studies as a substitute for double-hit cytogenetic status is not recommended. Although most DHL cases also exhibit double-expressor features, it is important to note that most DEL are not necessarily DHL.[11] In reported studies, DEL was observed more frequently in the ABC subtype, whereas DHL was more prevalent in the GCB subtype. Additionally, inferior overall survival (OS) and progression-free survival (PFS) were noted when c-MYC/BCL2 were co-expressed, but not with c-MYC expression alone by IHC.[12]

Aggressive lymphomas such as DHL and DEL require extensive diagnostic procedures for accurate identification and the utilization of expensive modern technology in subsequent monitoring. In a resource-poor country like the Philippines, access to this kind of technology is limited and entails large amounts of funding, and oftentimes, proper evaluation and diagnosis of this disease cannot be achieved fully which unfortunately, negatively impacts the patient's prognosis. Moreover, some pathologists in the country refrain from further subtyping this disease due to unavailability of the test and high cost. Consequently, clinicians tend to depend on the usual treatment regimen, even though existing literature

indicates that such treatments may prove ineffective for these types of lymphomas.

This study aimed to determine the clinicopathologic profile and over-all survival outcomes of Filipino patients with DLBCL admitted and managed in the Philippine General Hospital from 2016 to 2021. Additionally, this study also aimed to determine difference in over-all survival between double-hit versus nondouble-hit lymphoma and between double-expressor versus nondouble-expressor lymphoma, and to determine the concordance between double-hit as measured by FISH and double-expressor as measured by IHC.

METHODS

This study is a single-arm, retrospective cohort study, involving the cohort of patients with histopathologically diagnosed and subtyped DLBCL, from 2016 to 2021. The outcome of interest of the study is the over-all survival of the study participants, right-censored to the last known follow-up for patients without recorded mortality and for patients who were lost to follow-up. Additional co-variates of interest are the double-hit status and double-expressor status of the DLBCL.

Cases included were derived from the surgical pathology reports, both paper-format and electronic database (Open MRS), of the Department of Laboratories, University of the Philippines-Philippine General Hospital (PGH) released from January 1, 2016 to December 31, 2021 and have met all the following criteria: PGH patients 19 years old and above whose surgical pathology cases were signed out as NHL DLBCL, GCB, or non-GCB subtype with the aid of IHC studies. Excluded from this study were cases where paraffin blocks cannot be retrieved for additional IHC studies and FISH analysis. Additionally, cases with paraffin blocks that were less than 0.2 cm / 2.0 mm in thickness were not included due to possible consumption of tissue during processing. A review of the surgical pathology reports was done together with a hematopathologist. Only cases signed out as NHL, DLBCL, GCB or non-GCB subtype confirmed using an initial IHC panel composed of LCA, CD3, CD20, CD10, BCL6, and MUM1 were included. Cases signed out as NHL, DLBCL without subclassification, and incomplete IHCs were not included in the study. Data from the reports, including clinical information such as age at the time of diagnosis, gender, and the location of the tumor or mass, were also gathered for analysis. A chart review of the included cases was conducted with the following data collected: whether treatment was conducted in PGH, treatment protocol, presence or absence of B symptoms, serum LDH, IPI score, date of last follow-up/mortality, and whether the patient is alive or deceased based on their last follow-up and/or admission in PGH at the time of the study. Patients who did not receive treatment in PGH were excluded. Ensuring that all patients received treatment within the same institution helps maintain consistency in data collection and treatment administration, thereby reducing variability in the dataset. This approach strengthened the study by controlling for differences in treatment protocols, record-keeping, and follow-up procedures that might occur between different institutions.

Paraffin blocks of included patients were retrieved for a second panel of IHC studies which included c-MYC and BCL2.

FISH analysis was performed using tissue microarray. Probes specific for translocation detection involving loci of the MYC, BCL2, and/or BCL6 genes, following the manufacturer's instructions were used.

The IHC slides were reviewed together by the principal and co-investigator, a consultant and hematopathologist from the PGH Department of Laboratories. As a criterion for this investigation, both c-MYC and BCL2 must exhibit expression, categorizing them as DEL. Cut-off values are based on the WHO criterion: BCL2 is deemed positive if ≥ 50% of the tumor cells display staining, while c-MYC is considered positive if ≥ 40% of the tumor cells exhibit staining. Cases that did not exhibit staining for both c-MYC and BCL2, cases that stained with either c-MYC or BCL2 exclusively, and cases with staining less than 50% for BCL2 and/or less than 40% for c-MYC were classified as non-DEL.

The following DNA FISH probes were utilized in this study: ZytoLight SPEC MYC Dual Color Break Apart Probe (PL49), ZytoLight SPEC BCL6 Dual Color Break Apart Probe (PL136), and ZytoLight SPEC BCL2/IGH Dual Color Dual Fusion Probe (PL71). The number of tumor cells were counted and interpreted based on the cut-off by Ventura et al.[13]

The clinicopathologic characteristics of the cases were described. Quantitative variables were reported using mean and standard deviation (SD). Qualitative variables were presented as the actual count. Kaplan-Meier survival curves were generated to illustrate overall mortality in the entire cohort and to compare mortality between DEL and non-DEL, as well as DHL and non-DHL. Lastly, the concordance between FISH study and IHC testing results was determined.

Ethical Consideration

The study was accomplished after seeking ethical approval from the University of the Philippines Manila Research Ethics Board (UPMREB). A waiver of consent was requested and approved as there were no risks to the study participants.

RESULTS

Patient Characteristics

From 2016 to 2021, fifty-seven cases were diagnosed as NHL, DLBCL. Among these, only 29 cases had complete IHC stains for LCA, CD3, CD20, CD10, BCL6, and MUM1. Of these 29 cases, tissue blocks were still available for 17 cases at PGH, while the other 12 had been processed and returned to the patients or their relatives. Out of these 17 cases, only 14 were treated at PGH. Consequently, 14 cases met the inclusion criteria and were included in this study.

Table 1. Clinical Profile of Diffuse Large B Cell Lymphoma Patients in PGH Included in the Study

Patient profile	Full cohort n = 14	Double expressor		Double hit*	
		Yes n = 12	No n = 2	Yes n = 5	No n = 6
Age at diagnosis, years, mean (SD)	42.14 (13.40)	42.67 (12.02)	39 (26.87)	41.80 (14.01)	44.50 (11.74)
Sex, n					
Male	8	6	2	3	4
Female	6	6	0	2	2
Stage, n					
Stage I-II	2	2	0	0	2
Stage III-IV	12	10	2	5	4
With B symptoms, *n*	8	7	1	4	2
Serum LDH, n					
Normal	4	3	1	2	2
Elevated	10	9	1	3	4
IPI score, n					
Low (0-2)	5	4	1	2	3
High (3-5)	9	8	1	3	3
Cell of origin, n					
Germinal center	5	4	1	3	1
Non-germinal center	9	8	1	2	5
Location, n					
Nodal	5	4	1	2	3
Extranodal	9	8	1	3	3
Treatment protocol, n					
CHOP/RCHOP	7	5	2	2	4
R-EPOCH	7	7	0	3	2
Status					
Alive	9	7	2	3	3
Expired	3	3	0	1	2
Lost-to-follow up	2	2	0	1	1

** Only 11 cases have tissues still available for FISH testing to detect rearrangements involving MYC, BCL-2, and BCL-6.*

Fourteen cases met the inclusion criteria based on IHC and were included in this study. Most patients fell within the age group of 60 years old and below at the time of diagnosis, with an average age of 42.14 years and a standard deviation of 13.40 years. Most of the patients were males (8/14), and most exhibited at least stage III-IV disease (12/14), presenting with B symptoms (8/14) and elevated serum LDH (10/14) at the time of diagnosis (Table 1). Diagnosis was predominantly established from an extranodal location (9/14), and a significant number had a high IPI score (9/14). Approximately half of the patients received the standard CHOP/RCHOP chemotherapy regimen, while the other half was administered the R-EPOCH regimen. Most patients were alive (9/14) at the time of the study; three had passed away after completing a few cycles of chemotherapy, and two were lost to follow-up. Vital status was determined based on the time of initial diagnosis to their last known follow up in PGH.

Immunophenotypic Characteristics

The non-GCB subtype was more prevalent than the GCB subtype (9 vs. 5) when classified using the Hans' algorithm. Twelve cases (85.7%) were reported as DEL and two were non-DEL based on IHC studies. The mean age at diagnosis of the DEL cases was 42.67 years old with no sex predilection. Most DEL cases had at least stage III-IV disease (10/12), with B symptoms (7/12), and elevated serum LDH (9/12) at presentation. Majority were categorized as non-GCB COO (8/12) and was diagnosed extranodally (8/12). Seven were given the R-CHOP chemotherapy regimen and are still alive at the time of study.

Cytogenetic Characteristics (FISH Analysis)

FISH studies were conducted on eleven out of the fourteen DLBCL cases. Two DEL cases and one non-DEL case were excluded due to consumption of tissue in the paraffin block precluding further testing with FISH. Out of the eleven DLBCL cases, four DEL were verified as DHL, one non-DEL was classified as DHL, while the remaining six DEL were classified as non-DHL, resulting in a concordance rate of 36%. All DHL cases were diagnosed at age 60 years old and below. Most of the patients with DHL were males, with higher stage at time of presentation, experienced B

Table 2. Concordance between FISH (DHL) and IHC (DEL) Testing for Evaluating MYC, BCL-2, and BCL-6 Gene Rearrangements among Diffuse Large B Cell Lymphoma Patients

Double expressor	Double hit	
	Yes	No
Yes	4	6
No	1	0

Kappa statistic = -0.1846, 95% CI [-0.1957, 0.0000]

symptoms, with elevated LDH, diagnosed extranodally, and had high IPI scores. Majority of DHL cases were classified under GCB COO (Table 1).

Concordance between DEL and DHL

Table 2 shows the non-concordance between FISH and IHC testing when evaluating c-MYC, BCL2, and BCL6 gene rearrangements in DLBCL patients with a Kappa value of -0.1846, 95% CI [-0.1957, 0.0000].

Survival Curves

We determined the survival curve of the all the DLBCL cohort (Figure 1A). We also compared the survival curves of the DEL versus the non-DEL (Figure 1B) as well as the DHL from the non-DHL (Figure 1C). This was done using the Kaplan Meier survival curve. The survival curve for all DLBCL patients (Figure 1A) illustrates a plateau in the survivor function between three months and 37 months (probability = 0.8250). Significant difference was not identified because of the low sample size and relatively unbalanced distribution. Figure 1B illustrates survival curves of DEL versus non-DEL. All three fatalities were identified as cases of DEL. Figure 1C displays the survival curves comparing DEL to DHL.

DISCUSSION

On an annual basis, our institution typically diagnoses approximately 80-90 cases of NHL, specifically DLBCL. Ideally, before conclusively diagnosing DLBCL, the specimen should undergo a comprehensive set of tests, including a complete panel of IHC and molecular studies such as FISH. These tests are crucial to rule out other types of lymphomas and confirm the diagnosis of DLBCL. However, due to financial constraints or the limited availability of these tests, many clinicians forego ordering the ideal diagnostic tests. Instead, they often opt for an immediate trial of a chemotherapeutic regimen, which may be a hit or miss for the patient. Only fourteen (14) cases were included in the study. The limited number stems from the realization that a significant number of PGH patients could not afford the cost of a complete panel of IHC studies for subtyping to identify COO and additional IHC with c-MYC and BCL2 to identify DEL. As a result, these individuals will either be

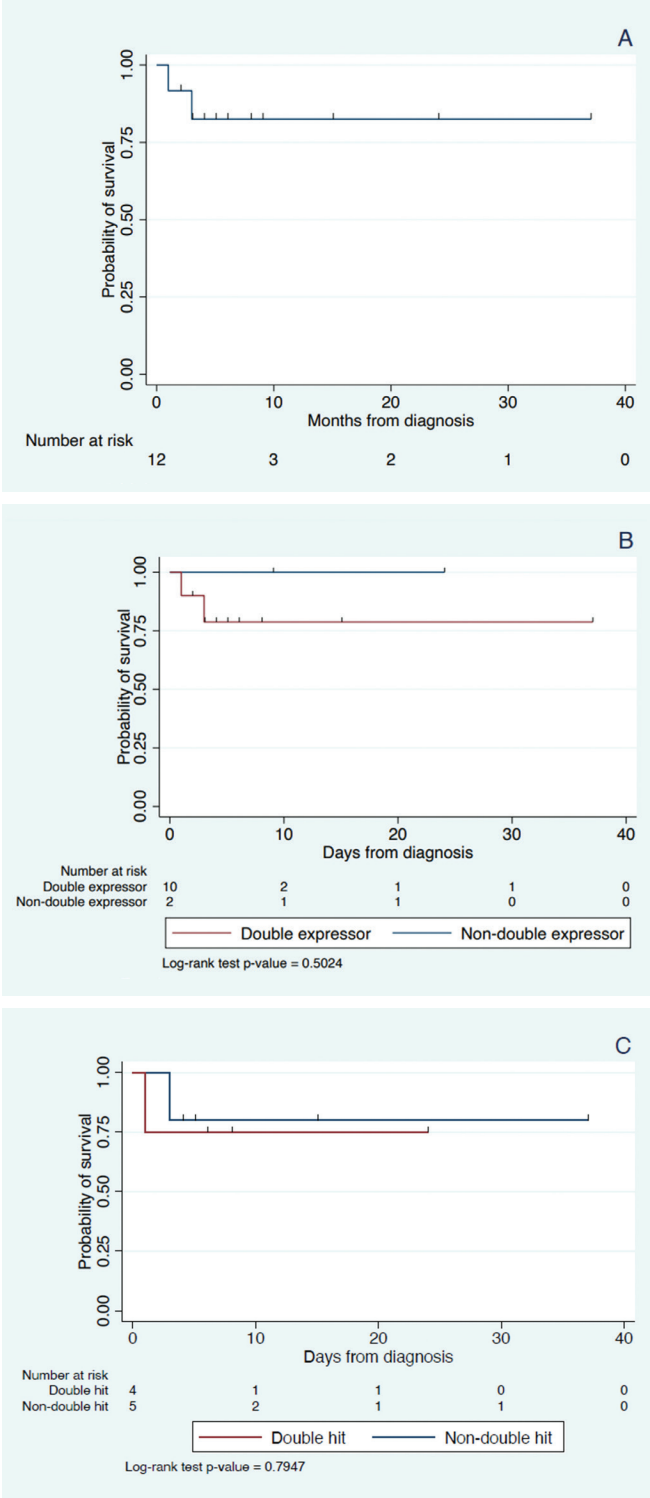

Figure 1. ***Kaplan-Meier survival curves of diffuse large B cell lymphoma patients in PGH included in the study.*** **(A)** Full cohort; **(B)** Comparison between double expressors and non-double expressors; **(C)** Comparison between double hit and non-double hit.

subjected to the routine DLBCL chemotherapeutic regimen or will be lost to follow-up without initiating any form of treatment. Furthermore, a significant number of individuals with the financial means to undergo the entire IHC stains panel chose to pursue chemotherapy at a facility of their choosing rather than at our institution. As such only a small number of cases remain within our institution, limiting the number of cases included in this study. Identifying DHL is also difficult without additional FISH analysis.[14] A diagnosis of DHL requires testing all aggressive lymphomas with FISH.[15] However, the high cost of this supplementary test can impose an additional financial burden on patients, as it is influenced by factors such as limited availability, costly reagents, the need for sensitive specimen storage, and expensive equipment. Judicious use of FISH testing through algorithmic approaches, especially in resource-poor areas, must be adopted. However, these too are not without limitations. We endeavored to establish a systematic approach for diagnosing DHL by examining clinical characteristics and immunophenotypic findings before conducting FISH analysis. However, the small sample size prevents us from determining its statistical significance, restricting our reporting to a total enumeration of the samples.

While more prevalent in the elderly population, the mean age of diagnosis, as reported in this study, is 42.14 years old. A significant majority of diagnoses occurred in individuals below the age of 60, contrasting with previously reported global data. Exact reasons cannot be deduced partly because of the small number of cases included in the study however, a possible reason may be attributed to the B symptoms experienced by patients, prompting them to seek medical consultation at an earlier age. Additionally, there is a slight male predilection (M: F, 1.33:1), aligning with previously reported global studies. Elevated LDH levels, high IPI scores, and presence of extranodal involvement seen in our study are also in agreement with previously reported cases. Majority of cases presented with a non-GCB COO, with reports demonstrating an inferior outcome for this subgroup. Although there may be variation in terms of the relative frequencies between GCB and non-GCB subtypes, it was found out that in most studies the non-GCB subtype predominates in Asians while c-MYC and BCL6 gene rearrangements still comprises majority of those with DHL.[16]

The correlation between FISH (DHL) and IHC (DEL) testing was examined (Table 2) to detect c-MYC, BCL2, and BCL6 gene rearrangements in DLBCL patients. The results indicate a lack of agreement between FISH (DHL) and IHC (DEL) testing when assessing c-MYC, BCL2, and BCL6 gene rearrangements among DLBCL patients. However, due to the limited sample size, its statistical significance cannot be deduced. Despite that, the findings in this study align with previously documented studies that emphasize the inability of IHC to replace FISH testing. This is because, besides gene rearrangement, gene amplification or mutation can also result in increased oncoprotein expression.[17] In DEL, c-MYC and BCL2 overexpression can also be attributed to gene amplification and posttranslational processes in the absence of chromosomal translocations (Riedel and Smith, 2018). In line with the 2016 WHO guidelines, despite individuals with DEL displaying a poor prognosis, the overexpression of c-MYC and BCL2 proteins cannot be used as a substitute indicator to determine whether DLBCL is a DHL. Therefore, FISH and/or other cytogenetic testing remain indispensable for an accurate assessment and diagnosis of DHL.

The survival curve for the entire cohort having a plateau between three months and 37 months is somewhat misleading due to the considerable number of patients that were lost to follow up (censored observations) within the first year after diagnosis. This may not truly reflect favorable survival but rather suggests challenges in patient retention. In Figure 1B, which compares the survival curves of DEL versus non-DEL, it is noteworthy that all three fatalities occurred in double expressors. However, due to the limited sample size and most of the observations being censored, we lack sufficient evidence to establish statistical significance (p-value = 0.5024). Figure 1C juxtaposes the survival curves of DHL versus non-DHL. Notably, the survival curves appear similar for double-hit (0.7500) and non-double-hit (0.8000) cases within the initial three months from diagnosis. Similarly, no statistically significant difference was identified.

Out of the 14 DEL cases initially included, only 11 cases met the criteria for additional FISH testing. The exclusion of three cases was due to suboptimal specimen conditions and consumption of tissue on processing. Among the qualified cases, five were identified as DHL, constituting approximately 45% of the total DLBCL cases subjected to FISH studies. This observation diverges from the prevalent trend in most studies, where roughly 5-12% of DLBCLs are typically reclassified as having double-hit/triple-hit status.[18] To definitively establish the statistical significance of this difference within the Filipino population, a more comprehensive, multi-center study with a larger sample size is essential. Various factors, including sampling bias, population disparities, patient demographics, and genetic predisposition, may contribute to the four-fold increase in the incidence of DHL seen in our institution. Further investigation into these factors is necessary to assert conclusively whether DHL indeed exhibits a higher incidence rate among the Filipino population.

CONCLUSION AND RECOMMENDATION

The clinicopathologic profiles of patients in our study are similar to those reported worldwide, with the exception of the younger age at diagnosis observed at this institution. Survival outcomes for patients with DLBCL ranges from three to 37 months. All cases of mortality were associated with DEL. In contrast, patients with DHL exhibited mixed vital status compared to those with non-DHL. Due to limited sampling, statistical significance of the results cannot be determined. A comprehensive diagnosis relies on an extensive panel of IHC

studies and FISH analysis. The variations observed in our sample population, when contrasted with global published reports, warrant further investigation to ascertain whether these differences are statistically significant and genuinely distinctive within the Filipino population.

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Statement of Authorship

All authors certified fulfillment of ICMJE authorship criteria.

Author Disclosure

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