CHAPTER I: Hematologic parameters and bone marrow flow cytometry features in patients with *GATA2* and *RUNX1* germline mutations

CHAPTER II: Evaluation of frequency of 11q aberrations in patients with Burkitt-like lymphoma and other aggressive B cell lymphomas: correlation with histopathology and clinical characteristics

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A thesis

submitted in partial fulfillment of the

requirements for the degree of

Master of Science

University of Washington

2021

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Program Authorized to Offer Degree:

Laboratory Medicine

University of Washington

Abstract

Hematologic parameters and bone marrow flow cytometry features in patients with *GATA2* and *RUNX1* germline mutations

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For patients presenting with cytopenias, the clinical presentation and histopathological distinction between an inherited (germline) bone marrow failure syndrome and an acquired bone marrow failure/myelodysplastic syndrome (MDS) can be challenging. Prompt and precise diagnosis of is critical to inform appropriate management before a leukemic transformation. *GATA2* and *RUNX1* are known germline pre-disposition mutations affecting master transcription factors important in lineage development. Here we aimed to develop a screening assay to help assess patients found to have germline *GATA2* mutation and assist in germline variant curation. We compared the hematologic parameters and early bone marrow flow cytometric features in 8 patients with germline *GATA2* mutations to 15 patients with aplastic anemia at our institution. To further consider the specificity of the findings in *GATA2* deficiency patients, we compared their findings to 9 patients with germline *RUNX1* mutations. Consistent with prior studies, our patients with *GATA2* mutations showed less pronounced anemia and thrombocytopenia than those with aplastic anemia. Findings in the marrow, including the absence of hematogones, and reduction in NK cells, also support prior studies. Furthermore, compared to patients with *RUNX1* mutations, patients with *GATA2* mutations showed significantly lower B cells (of lymphocytes) and NK cells (of lymphocytes), and higher T cells (of

lymphocytes). Interestingly, we also identified patients with *RUNX1* mutations to have significantly more PDCs than patients with *GATA2* mutations. Differences in our findings from prior studies, including similar peripheral blood absolute white counts, absolute neutrophils, absolute monocytes, absolute lymphocyte counts, and B cells or monocytes in the marrows of our patients with germline *GATA2* mutations, are due to the small size of patient cohorts given the rarity of these germline variants as well as to variable disease status at presentation for evaluation/inclusion in these studies (i.e., pre-MDS, MDS). The combined findings contributed to the understanding of how these mutations establish variable pre-leukemic states predisposing to myeloid malignancies and supported the development of effective screening strategies.

University of Washington

Abstract

Evaluation of frequency of 11q aberrations in patients with Burkitt-like lymphoma and other aggressive B cell lymphomas: correlation with histopathology and clinical characteristics

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Burkitt-like lymphoma with 11q aberration (BLL-l1q) is one of the newly described provisional entities with morphologic features, immunophenotype, and gene expression profile similar to Burkitt lymphoma (BL) but lacks *MYC* rearrangement. Whether this entity is a distinct category or a variant of BL, diffuse large B cell lymphoma (DLBCL), or high-grade B cell lymphoma is still controversial. Few cases have been reported, leading to poor understanding of clinical presentations, treatment regimens, or full understanding of the landscape of mutations/copy-number alterations in BLL-11q. Here we aimed to evaluate the frequency of 11q aberration in our patients and their histopathologic characteristics, clinical presentations, and responses to therapy received. Thus, we performed genomic array on 10 BL patients in all ages, 5 DLBCL or HGBCL patients with CD10 or BCL6 positive and BCL2 negative under 60 years old ("Burkitt-like" group), and 4 DLBCL or HGBCL patients with CD10 or BCL6 positive and BCL2 positive under 60 years old. No typical 11q gain/loss pattern were found in our patients according to WHO classification at this time point. One patient in the BL group was identified with 11q terminal deletion, which is within the regions identified by prior BLL-11q studies. In addition to 11q aberrations, other findings in the BL group and the

DLBCL or HGBCL with BCL2+ group further reflect previous studies on patients with BL and DLBCL. We concluded that the groups of patients are currently too limited for meaningful statistical assessment of the frequency of 11q aberrations, but suggest that 11q aberrations are not highly frequent in our “Burkitt-like” cases. We also observed the limitations on performing genomic array on older FFPE samples. Our future directions include requesting and running more recent samples, using stored RNA for transcriptome, and considering other significant findings in Burkitt-like patients without 11q aberrations.

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Chapter I. Hematologic parameters and bone marrow flow cytometry features in patients with *GATA2* and *RUNX1* germline mutations

Section 1. Introduction

For patients presenting with cytopenias, the clinical presentation and histopathological distinction between an inherited (germline) bone marrow failure syndrome and an acquired bone marrow failure/myelodysplastic syndrome (MDS) can be challenging. The precise identification of inherited syndromes is critical to inform appropriate clinical management, including timely hematopoietic stem/progenitor cell transplantation (HSCT) and identifying potential donors (1–3). This need is emphasized by the recent inclusion of myeloid neoplasms with germline predisposition category into the revised fourth edition of the World Health Organization (WHO) Classification of Tumours of Haematopoietic and Lymphoid Tissues (4). Given this emphasis, clinicians and laboratories are more frequently identifying, and reporting, germline variants, some of which are reported as variants of uncertain significance (VUS). When germline mutations are identified, and particularly when a VUS is identified, patients, family members, and clinicians look for reliable methods for predicting the presence, and pathogenicity, of the identified mutation, to allow for surveillance and appropriate timing of therapeutic intervention.

Many of the known germline pre-disposition mutations affect master transcription factors important in lineage development, the same genes seen with somatic mutations in MDS/acute myeloid leukemia (AML), such as *GATA2* and *RUNX1*. Germline *GATA2* mutations, causing haploinsufficiency, lead to cellular immunodeficiencies, higher susceptibility to disseminated non-tuberculous mycobacterial or human papillomavirus infections, and increased risk of developing myeloid malignancies, such as AML (1,5). One recent study sought to evaluate bone marrow flow cytometric, morphologic, and cytogenetic features in 28 patients with germline *GATA2* mutations, finding severely reduced monocytes, B cells, and NK cells, absent hematogones, and inverted CD4:CD8 ratios. Germline mutations in *RUNX1* define familial platelet disorder with

predisposition to myeloid malignancy, characterized by abnormalities in platelet counts and/or function but with normal platelet size, and increased prevalence of early-onset MDS/AML and T-lymphoblastic leukemia/lymphoma. Prompt diagnosis of these germline disorders can support appropriate clinical interventions before leukemic transformation (6–8).

Here, we sought to assess hematology parameters and early bone marrow flow cytometric features in patients with germline *GATA2* mutations at our institution, and compare these findings to patients with acquired aplastic anemia. To further consider the specificity of the findings in GATA2 deficient patients, we also sought to compare findings in the *GATA2* group of patients to a group of patients at our institution with germline *RUNX1* mutations, evaluated prior to onset of leukemia. We aimed to identify features/diagnostic "clues" to distinguish these presentations and assist in germline variant curation, and to confirm the peripheral blood and marrow features from the prior studies of moderately sized groups of patients with these mutations.

Section 2. Materials and Methods

Patients

Three groups of patients were included in this retrospective case-control study. The first group, referred to hereafter as, "control" group, consisted of 15 patients who underwent bone marrow evaluation for clinical work-up of unexplained cytopenias, and were clinically diagnosed with aplastic anemia. All patients in this group showed response to immunosuppressive therapies; the majority were also evaluated by a germline molecular panel as part of clinical evaluation and all of those evaluated by the panel showed lack of identifiable germline mutations (including *GATA2* and *RUNX1* mutations). The second group included 8 patients with germline *GATA2* mutations. The third group consisted of 9 patients with germline *RUNX1* mutations. Additional details including age, gender, clinical and histopathology diagnosis, severity assessment by the modified Camitta criteria (9) (for patients with aplastic anemia) and complete blood count values are summarized in Table 1. Patients with aplastic anemia had a median age of 27 years old (range 10-69 years), patients with germline *GATA2* mutations had a median age of 25 years old (range 10-44 years), and patients with germline *RUNX1* mutations had a median age of 37 years old (range 26-42 years).

Mutation evaluation

Mutation profiling was performed by hybrid capture next-generation based assays available at our institution (MarrowSeq, Heme Capture) on skin fibroblasts and/or bone marrow samples. Details of these assays have been described previously (10).

Flow cytometry analysis

Flow cytometry studies were performed by retrospective analysis of data from routine clinical panels (11). In brief, samples were analyzed using four clinically validated reagent combination tubes, one focused on B cells (containing fluorochrome-conjugated antibodies targeting CD5, CD19, CD20, CD38, CD10, CD45, kappa, lambda), one focused on T cells (containing anti-CD2, CD3, CD4, CD5, CD7, CD8, CD30, CD34, CD45, CD56), and two focused on identification of myelomonocytic populations (combinations of anti-CD4, CD13, CD14, CD15, CD16, CD19, CD33, CD34, CD38, CD45, CD71, CD117, CD123, and HLA-DR). At least 200,000 viable cell events were evaluated per tube. For the purposes of this study, we re-gated populations using similar gating strategy to prior publications on patients with germline *GATA2* mutations (12). Lymphocytes were gated by CD45 and SSC expression. We then defined hematogones as $CD19^{+}CD10^{+}CD20^{variable}$, mature B cells as $CD19^{+}CD10^{-}CD20^{+}$, natural killer (NK) cells as $CD3^{-}CD7^{+}$ lymphoid cells; T cells by CD3 expression, dendritic cells by $CD123^{+}$HLA-DR^{+}, and monocytes by CD33, HLA-DR, and level of SSC expression. Data analysis was performed by an in-house developed software (WoodList version 3.1).

Statistical evaluation

Unpaired, nonparametric t tests (Mann-Whitney t tests), were used to compare differences between groups. A p-value less than 0.05 was considered statistically significant. Statistical analyses and graph generation were performed using GraphPad Prism software (version 9.1.2).

Section 3. Results

Patients with germline *GATA2* mutations demonstrate less pronounced anemia and thrombocytopenia than patients with aplastic anemia

The following data is additionally displayed in Table 1 and Table 2. We identified peripheral blood CBC data (concurrent to the bone marrow flow cytometry study) for 8 of the patients with germline *GATA2* mutations, 9 patients with germline *RUNX1* mutations, and 15 patients with aplastic anemia. Of the 8 patients with *GATA2* germline mutations, 4 were diagnosed in that marrow sampling with myelodysplasia (three with monosomy 7, one with del5q) by bone marrow morphologic evaluation and cytogenetics/standard MDS FISH panel. Patients with *GATA2* germline mutations presented with variable cytopenias, most frequently leukopenia (7 of the 8 patients). Four of the 8 patients with germline *GATA2* mutations were pancytopenic, 2 demonstrated bi-cytopenia, 1 patient demonstrated only thrombocytopenia, and 1 patient only leukopenia. In contrast, 12 of the 15 patients with aplastic anemia demonstrated pancytopenia. Based on comparison of the CBC values, patients with germline *GATA2* mutations were overall noted to have less pronounced anemia and less pronounced thrombocytopenia in comparison to patients with aplastic anemia (for hemoglobin: $p=0.003$, for platelet count: $p<0.001$) **(Figure 1A and 1B)**. Absolute white counts, absolute neutrophil counts, absolute monocyte counts, absolute lymphocyte counts, and MCV were not significantly different between patients with germline *GATA2* mutations and those with aplastic anemia **(Figures 1C, 1D, 1E and 1F; MCV data not shown) (Table 2).** Marrow cellularity was significantly lower in patients with aplastic anemia (mean 18%, median 20%, range 5-40%), in comparison to those was with germline *GATA2* mutations (mean 38%, median 40%, range 15-70%) ($p = 0.008$).

Patients with *RUNX1* mutations showed fewer cytopenias than patients with *GATA2* mutations or aplastic anemia. None of the patients with germline *RUNX1* mutations were pancytopenic at the time of evaluation (1 had leukopenia, and 7 of 8 had mild thrombocytopenia). Patients with *GATA2* mutations were found to have significantly lower white cell counts (*p=0.003*) **(Figure 1C)** and lower absolute neutrophil counts (*p=0.002*) **(Figure 1D)** than patients with *RUNX1* mutations. Similar, but more pronounced, findings were detected between patients with *RUNX1* mutations and those with aplastic anemia. In comparison to patients with *RUNX1* mutations, patients with aplastic anemia showed lower hemoglobin (*p<0.001)*, lower platelet counts *(p=0.001)*, lower absolute WBC counts (*p<0.001)*, lower absolute neutrophil counts *(p<0.001)*, lower absolute monocyte counts *(p=0.001)*, and lower absolute lymphocyte counts (*p=0.001)* (**Figures 1A, 1B, 1C, 1D, 1E,** and **1F**). All patients with germline *RUNX1* mutations showed normocellular or mildly hypocellular marrows (mean cellularity 58%, median 60%), which was significantly more cellular than marrows from patients with *GATA2* mutations (*p = 0.01)* and marrows from patients with aplastic anemia (*p <0.001).*

Patients with *GATA2* mutations demonstrate nearly absent hematogones, while those with *RUNX1* show more frequent hematogones and CD34 positive myeloid progenitors

Summary of findings from the retrospective review of bone marrow flow cytometry studies is shown in Table 3. Patients with *GATA2* mutations were found to have nearly absent hematogones, consistent with previous reports (12,13). This finding was statistically significant in comparison to patients with aplastic anemia as well as in comparison to patients with *RUNX1* mutations (*GATA2* v AA: *p<0.001; GATA2* v *RUNX1*: *p<0.001*) **(Figure 2A)**. Differences were

significant calculated as percent of lymphocytes or as percent of total WBC (for WBC, *GATA2* v AA: *p<0.001; GATA2* v *RUNX1*: *p<0.001,* data not shown).

All patients included in our study showed less than 5% bone marrow blasts by morphology; CD34 by immunohistochemistry (IHC) was not performed in all cases, limiting comparison of CD34 counts by IHC. CD34 progenitor percentage (as measured by flow cytometry, after erythroid lysis) showed slightly fewer CD34 positive progenitors in patients with aplastic anemia than in patients with germline *GATA2* mutations (aplastic anemia median 0.10%; *GATA2* median 0.45%; *p = 0.02*) **(Figure 2B)**. Patients with *RUNX1* germline mutation demonstrated the highest frequency of CD34 positive progenitors of the groups (*RUNX1* median 1.04%, *p value RUNX1 v GATA2 p =0.01, p value RUNX1 v AA: p<0.001)* (**Figure 2B**). We did not observe significant differences in the proportion of CD117 positive progenitors between any of the three groups of patients (**Figure 2C**).

Patients with germline GATA2 mutations demonstrate fewer NK cells and nearly absent PDCs, while patients with germline RUNX1 mutations show a proportionate increase in PDCs.

Despite the near absence of hematogones, in our data patients with *GATA2* mutations demonstrated a similar proportion of total CD19 positive B cells in the marrow in comparison to control patients (**Figure 2D**), and a mild proportionate increase of T cells (*p=0.014)*. *RUNX1* patients were interestingly found to have more B cells in the marrow in comparison to patients with *GATA2* mutations (*p<0.001*) or aplastic anemia (*p=0.015)* (**Figure 2D),** and fewer T cells (**Figure 2E)**. We identified no significant difference in the CD4:CD8 ratio of T lymphocytes between the three groups in the marrow (data not shown).

Prior studies have demonstrated decreased NK cells in patients with germline *GATA2* mutations. Similarly, in our study, *GATA2* patients demonstrated fewer NK cells than patients with aplastic anemia (*p=0.008*) or patients with *RUNX1* mutations (*p=0.008*) *(***Figures 2F**). Interestingly, despite the peripheral blood finding of relatively increased peripheral monocytes in *RUNX1* patients, and in contrast to prior studies showing decreased marrow monocytes in patients with *GATA2* mutations, we did not see significant differences in the proportion of total bone marrow monocytes between any of the three groups (**Figures 2G**).

Recent studies have highlighted proliferative effects of *RUNX1* mutations in myeloid neoplasms leading to expansions of plasmacytoid dendritic cells (PDCs)(14). Given inclusion of markers allowing general identification of these cells in our panels (CD123+, HLA-DR+), we sought to evaluate the frequency of PDCs in our patient groups. Patients with *GATA2* mutations demonstrated nearly absent PDCs, while patients with *RUNX1* mutations demonstrated significantly increased proportion of PDCs in comparison to *GATA2* (*p<0.001)* and aplastic anemia patients (*p=0.01*) (**Figure 2H**).

Section 4. Discussion

Germline *GATA2* deficiency represents one of more than 300 genetic disorders associated with primary immunodeficiency affecting various components of the immune system. With recent advances in genomics, and increased frequency of genetic testing, it is of interest to have supplementary tests available to analyze the hematopoietic system, quantitatively and functionally, to correlate clinical phenotype with genotype and monitor patients with detected germline mutations.

Our study sought to evaluate hematopoietic populations in patients presenting with cytopenias and germline *GATA2* mutations, assessing for a significant "signature" of features that could assist in qualitative assessment for patients with identified germline *GATA2* mutations. In assessing for this "signature" of features, we chose to compare our *GATA2* group of patients with a group of patients diagnosed with aplastic anemia, negative for mutations in *GATA2* (or other mutations in our institution's germline panel). Furthermore, to consider the specificity of the findings in GATA2 deficient patients, we also sought to compare findings in the *GATA2* group to a group of patients at our institution with germline *RUNX1* mutations.

Prior studies have shown relatively reduced monocyte and lymphocyte counts, and higher platelet and absolute neutrophil counts, in patients with germline *GATA2* mutations than those with aplastic anemia (12). Our patients with *GATA2* mutations did show less pronounced thrombocytopenia than those with aplastic anemia; however, in our data both groups showed similar peripheral blood absolute white counts, absolute neutrophils, absolute monocytes, and absolute lymphocyte counts. Our study is limited by lack of peripheral blood flow cytometry data to provide more detailed assessment of circulating populations.

Several of our findings in the marrow, including absence of hematogones, and reduction in NK cells, support the findings of other prior studies on patients with germline *GATA2* mutations (12,13,15). Interestingly, in contrast to prior studies, we did not identify reductions in B cells or monocytes in the marrows of our patients with germline *GATA2* mutations (13,15). The reason for the discordance with prior testing is not likely to be due to differences in flow cytometry gating strategy as we used similar approaches to the prior study for defining each population.

These differences in both peripheral blood populations and marrow populations from prior studies may be due to small size of patient cohorts in these studies given the relative rarity of these germline variants as well as to variable disease status at presentation for evaluation/inclusion in these studies (i.e., pre-MDS, MDS). Clarity is likely to be gained from subsequent larger studies as these patients are increasingly more recognized and evaluated at various stages of pathogenesis.

Mutations in *GATA2* have been shown to effect changes in hematopoietic survival and cell differentiation (2,5). To consider the specificity of our findings to *GATA2* mutations, we compared our findings to data available for a group of patients at our institution with germline *RUNX1* mutations which also predisposes patients to myeloid malignancies. The patients with germline *RUNX1* mutations showed significantly higher B cells (of lymphocytes) and NK cells (of lymphocytes), and fewer T cells (of lymphocytes), in the marrow than patients with *GATA2* mutations. While all patients in the groups included in the study showed less than 5% blasts in the marrow, those with *RUNX1* showed proportionately more CD34 positive progenitors than patients with *GATA2* mutations or aplastic anemia. Interestingly, we also found significantly more PDCs in the patients with *RUNX1* mutations, in contrast to relatively rare PDCs in patients with *GATA2* mutations. Evaluation of dendritic cell populations in patients with myeloid neoplasms is an active area of investigation. Reductions in dendritic cell populations has been described in murine studies

of *GATA2* mutations as well as a small cohort of patients with germline *GATA2* mutations (15–17). PDC expansions in patients with *RUNX1*-mutated myeloid stem cell neoplasms has been a more recent active area of interest, provisionally affecting classification of myeloid neoplasms (4,14).

The combined findings support different roles of GATA2 and RUNX1 proteins in hematopoiesis in patients with germline variants. Studies of these patient populations assist in understanding how these mutations establish variable pre-leukemic states predisposing to myeloid malignancies, supporting development of effective evidence-based screening and treatment strategies for these patients.

								Peripheral Blood Count						
Patient	Cause of marrow failure	Bone marrow pathologic diagnosis	Disease severity by Camitta criteria [3]	Age	Marrow cellularity (%)	CD34+ % (marrow)	Gender	White Count (THOU/µL)	Hemoglobin (g/dL)	MCV (fL)	Platelet Count (THOU/µL)	Neutrophil Count (THOU/µL)	Monocyte Count (THOU/µL)	Lymphocyte Count (THOU/µL)
1	GATA2 deficiency	Mildly hypocellular marrow for age		44	40	0.46	F	3.78	13.3	93	178	2	0.04	1.66
2	GATA2 deficiency	MDS with del5q		10	15	0.12	F	2.84	10	91	72	2.12	0.01	0.65
3	GATA2 deficiency	Hypocellular marrow for age		22	20	0.26	F	3.53	11.4	93	224	1.34	0	2.01
4	GATA2 deficiency	Hypocellular marrow for age		42	40	3.86	F	1.58	8.2	111	71	0.65	0.08	0.83
5	GATA2 deficiency	MDS with monosomy 7		23	20	0.43	M	1.85	12.2	103	94	0.3	0.04	1.49
6	GATA2 deficiency	MDS with monosomy 7		40	60	0.40	F	3.94	11.5	96	149	1.93	0.55	1.18
7	GATA2 deficiency	MDS with monosomy 7		26	40	0.64	M	3.32	14.6	99	92	1.64	0.13	1.53
8	GATA2 deficiency	Normocellular marrow for age		17	70	0.54	M	8.16	15.8	91	110	3.92	1.14	2.69
10	FPD/PMM (RUNX1) [1]	Normocellular marrow for age		39	60	1.18	F	8.86	12.8	81	166	4.87	0.62	2.48
11	FPD/PMM (RUNX1)	Normocellular marrow for age		26	60	1.85	F	7.61	11.9	86	145	4.49	0.76	2.21
12	FPD/PMM (RUNX1)	Normocellular marrow for age		28	60	1.04	F	6.66	13.9	86	132	3.66	0.47	2.33
13	FPD/PMM (RUNX1)	Mildly hypocellular marrow for age		37	40	0.79	M	4.16	14.3	84	94	2.25	0.46	1.33
14	FPD/PMM (RUNX1)	Normocellular marrow for age		37	60	0.82	F	5.37	12.9	87	97	3.17	0.27	1.88
34	FPD/PMM (RUNX1)	Normocellular marrow for age		35	60	2.00	F	6.18	14.5	84	142	4.45	0.25	1.42
35	FPD/PMM (RUNX1)	Normocellular marrow for age		37	60	0.82	F	5.63	13.8	91	145	2.99	0.37	1.91
37	FPD/PMM (RUNX1)	Normocellular marrow for age		40	60	0.55	F	8.58	12.9	76	145	4.81	0.6	2.57
38	FPD/PMM (RUNX1)	Normocellular marrow for age		42	65	1.58	M	6.79	16	86	127	3.73	0.34	2.58
15	Acquired aplastic anemia	Hypocellular marrow for age	Non-severe	24	10	0.01	F	5	6.4	96.2	16	3.14	0	1.45
16	Acquired aplastic anemia	Hypocellular marrow for age	Non-severe	20	30	0.32	M	3.4	10.4	90	40	1.09	0.54	1.74
17	Acquired aplastic anemia	Hypocellular marrow for age	Non-severe	22	30	1.40	M	0.92	7.4	89	248	0	0	0.49
18	Acquired aplastic anemia	Hypocellular marrow for age	Non-severe	27	20	0.45	F	2.78	12.2	110	45	1.42	0.22	1.11
20	Acquired aplastic anemia	Hypocellular marrow for age	Non-severe	22	20	0.86	M	3.74	11.5	97	46	1.94	0.45	1.31
21	Acquired aplastic anemia	Hypocellular marrow for age	Severe	22	20	0.18	M	3.65	9.1	99	15	0.88	0.22	2.51
22	Acquired aplastic anemia	Hypocellular marrow for age	Very severe	56	5	0.02	F	0.72	12.3	90	10	0.01	0	0.71
23	Acquired aplastic anemia	Hypocellular marrow for age	Non-severe	40	5	0.00	F	3.49	8.2	91	3	1.33	0.17	1.99
27	Acquired aplastic anemia	Hypocellular marrow for age	Very severe	41	10	0.02	M	0.33	8.1	88	8	0.01	0.01	0.29
29	Acquired aplastic anemia	Mildly hypocellular marrow for age	Non-severe	33	40	0.28	F	3.99	10.3	103	38	2.55	0.4	1
30	Acquired aplastic anemia	Mildly hypocellular marrow for age	Non-severe	10	40	0.27	F	1.77	7.9	78	24	0.37	0.07	1.28
41	Acquired aplastic anemia	Hypocellular marrow for age	Non-severe	26	5	0.08	F	2.18	9.5	91	44	0.28	0.07	1.83
42	Acquired aplastic anemia	Hypocellular marrow for age	Non-severe	69	5	0.07	F	4	8.1	98	17	1.2	0.24	2.36
43	Acquired aplastic anemia	Hypocellular marrow for age	Very severe	34	5	0.05	F	0.42	9.3	82	5	0	0	0.38
44	Acquired aplastic anemia	Hypocellular marrow for age	Severe	51	20	0.10	M	1.84	6.8	89	17	0.45	0.14	1.21

[1] FPD/PMM refers to Familial Platelet Disorder with Propensity to Myeloid Malignancy
[2] CBC data timepoint correspond to date of evaluation
[3] Camitta BM, Rappeport JM, Parkman R, Nathan DG. Selection of patients for bone marrow transplantation in severe aplastic anemia. Blood. 1975 Mar;45(3):355–63.

TABLE 1: Clinical indication for evaluation and complete blood count results.

Eight patients with *GATA2* germline mutation, nine patients with *RUNX1* germline mutation, and fifteen patients with aplastic anemia without identifiable germline mutations are shown in the table. Reference ranges: white cell count (4.3 - 10 THOU/µL), Neutrophil Count (1.8 - 7 THOU/µL), Monocyte Count (0 - 0.8 THOU/µL), Lymphocyte Count (1.0 - 4.8 THOU/µL), MCV (81 - 98 fL), Hemoglobin (11.5 - 15.5 g/dL(female), 13.0-18.0 (male)), Platelet Count (150 - 400 THOU/µL).

CBC features	GATA2	RUNX1	AA	P (GATA2 vs AA)	P (RUNX1vs AA)	P (GATA2 vs RUNX1)
Hemoglobin (median, g/dL)	11.85	13.8	8.2	0.003	<0.001	0.174
Platelet Count (median, THOU/µL)	102	142	17	<0.001	0.001	0.496
White Count (THOU/µL)	3.425	6.66	2.18	0.357	<0.001	0.003
Neutrophil Count (THOU/µL)	1.785	3.73	0.59	0.100	<0.001	0.002
Monocyte Count (THOU/µL)	0.06	0.46	0.1	0.987	0.001	0.044
Lymphocyte Count (THOU/µL)	1.51	2.21	1.31	0.875	0.013	0.075

Table 2: Summary of CBC features of peripheral blood from patients with *GATA2* mutation, *RUNX1* mutation, and aplastic anemia

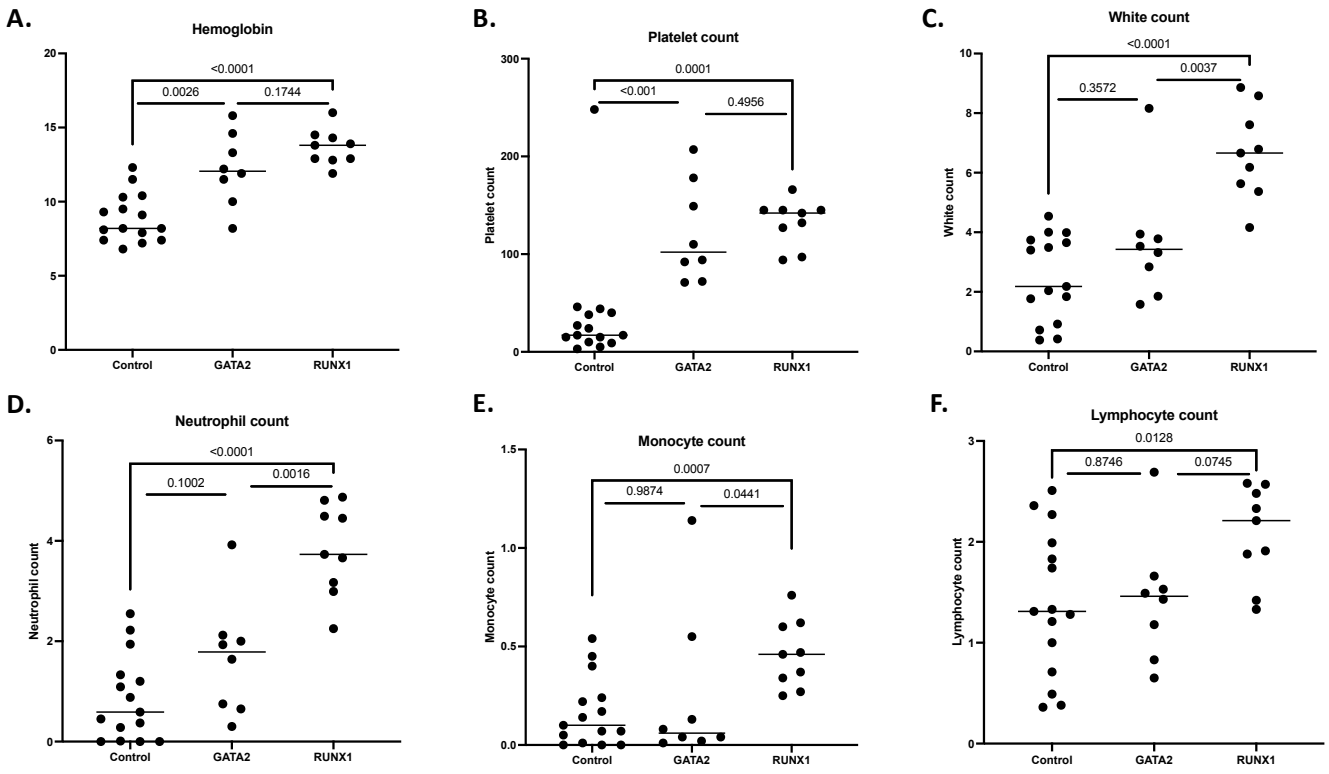

Figure 1: Peripheral blood CBC parameter in patients with aplastic anemia, *GATA2* germline mutations, and *RUNX1* germline mutations.

Hematologic peripheral blood parameters are compared between patients with aplastic anemia (n=15), *GATA2* germline mutations (n=8) and *RUNX1* mutations (n=9). Parameters evaluated include hemoglobin (A), platelet counts (B), absolute white cell counts (C), absolute neutrophil counts (D), absolute monocyte counts (E), and absolute lymphocyte counts (F). P-values (Student's t-test) are shown between groups.

Bone marrow flow cytometry features	GATA2	RUNX1	AA	P (GATA2 vs AA)	P (RUNX1vs AA)	P (GATA2 vs RUNX1)
Hematogone (median, %lymphocytes)	0	20.315	2.58	<0.001	0.013	<0.001
CD34+ progenitor (median, %WBC)	0.447	1.037	0.103	0.024	<0.001	0.011
CD117+ progenitor (median, %WBC)	0.719	1.79	0.63	0.636	0.193	0.37
B cell (median, %lymphocytes)	9.22	23.208	15.574	0.095	0.015	<0.001
T cell (median, %lymphocytes)	77.818	37.32	66.736	0.014	<0.001	<0.001
NK cell (median, %lymphocytes)	2.567	1.996	5.034	0.008	0.6	0.008
Monocyte (median, %WBC)	3.545	6.627	5.472	0.729	0.194	0.37
Dendritic cell (median, %WBC)	0.008	0.41	0.14	0.016	0.01	<0.001

Table 3: Summary of flow cytometry features of BM aspirates from patients with *GATA2* mutation, *RUNX1* mutation, and aplastic anemia

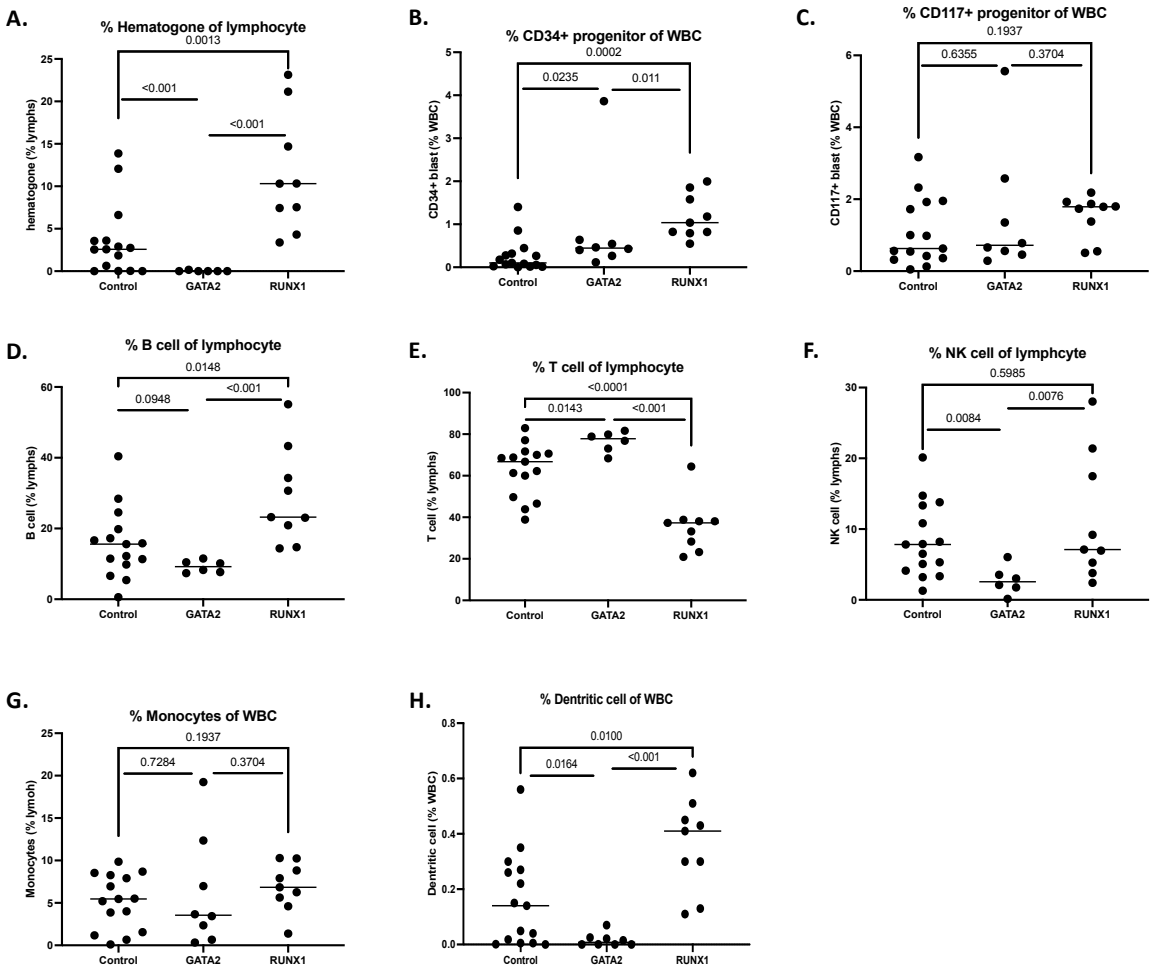

Figure 2: Comparison of proportions of progenitors and mature cell populations in bone marrow of patients with aplastic anemia, germline *GATA2* mutations, and germline *RUNX1* mutations.

Proportions of bone marrow progenitors (CD34+ and CD117+) and mature cells populations are compared between patients with aplastic anemia (n=15), *GATA2* germline mutations (n=8) and *RUNX1* mutations (n=9). Parameters evaluated include hematogones (of lymphocytes) (A), CD34+ progenitors (of WBC) (B), CD117+ progenitors (of WBC) (C), B cells (of lymphocytes) (D), T cells (of lymphocytes) (E), NK cells (of lymphocytes) (F), monocytes (of WBC) (G), and dendritic cells (of WBC) (H). P-values (Student's t-test) are shown between groups.

Chapter II. Evaluation of frequency of 11q aberrations in patients with Burkitt-like lymphoma and other aggressive B cell lymphomas: correlation with histopathology and clinical characteristics

Section 1. Introduction

Introduction

Within the past few years, due to the rapid development of genomic analysis, our understanding of lymphomas has improved dramatically. In particular, additional genomic understanding has led to further distinctions of the more aggressive B cell lymphomas (i.e., Burkitt lymphoma (BL), high grade B cell lymphomas (HGBCL), and diffuse large B cell lymphoma (DLBCL)). One of the new provisional entities incorporated in the revised 4th edition of the *World Health Organization (WHO) classification of Tumours of Haematopoietic and Lymphoid Tissue* is "Burkitt-like lymphoma with 11q aberration" (BLL-l1q). Whether this entity is a distinct category or a variant of BL, DLBCL, or HGBCL, is still controversial. Per the WHO provisional definition, BLL-11q is similar to BL in morphologic features, immunophenotype, and gene expression profile, but lacks the 1q gain frequently seen in BL and lacks *MYC* rearrangement. BLL-11q cases have 11q aberrations, most frequently described as consisting of a proximal gain in 11q21-23.3 and a telomeric loss of 11q 24.1-ter (4).

Recently, genomic aberration and transcriptomic studies on BL and DLBCL have defined differences in the mutation/copy number and transcriptome profile between these entities more clearly. Some studies suggest the transcriptome in Burkitt 11q is more similar to DLBCL than to BL (18), others proposed that it is distinct from BL or DLBCL/FL (19). However, overall, relatively few numbers of BLL-11q cases have been reported, leading to poor understanding of clinical presentations, treatment regimens, or full understanding of the spectrum of associated mutations/copy-number alterations in BLL-11q. Importantly, the most appropriate treatment regimen for BLL-11q is still unclear. Some of the patients were reported to respond well to BL regimens, while others were reported to respond to DLBCL regimens (18,20).

The clarification of biological characteristics of this new and rare subtype of lymphoma is clinically significant due to the increasing interest in determining the most appropriate management procedure in pediatric and young adult patients of this lymphoma. On the other hand, molecular and transcriptome analysis is expensive and time consuming for a clinical laboratory, and requiring inclusion of such findings may delay sign out/diagnosis of cases. It is therefore critical to determine the specificity of such aberrations, and consider carefully any real prognostic impact. Therefore, we aimed to improve the understanding of BLL-11q in our patients by assessing for the specificity of the 11q aberration by evaluating for the frequency of 11q aberrations and other copy number alterations in patients clinically diagnosed with Burkitt lymphoma and diffuse large B cell lymphoma (germinal center subtype, BCL2+ or BCL2-), under 60 years of age. Upon identifying patients with 11q aberrations, we then aimed to evaluate the histopathologic characteristics of the BLL-11q cases, clinical presentations, and responses to therapy received.

Literature review

Until now, around 10 - 15 prior studies or case reports have been published on BLL-11q (several of the published studies include overlapping patients). Before the WHO recognized it as BLL-11q, one study, Salaverria et. Al (2014), was evaluating the frequency of 11q aberration in their patients. They presented three cohorts in their study. The first cohort, including 59 cases classified by the study as “BL cases”, two were found to have no *MYC* translocation, gains of 11q22-q24, losses of 11q24-qter, and showed 9 and 12 CNAs, respectively. In cohort 2, consisting of 12 cases and additional two cell lines of HGBCL with features of BL as well as *MYC* rearrangement negative, terminal losses were seen in all of the samples and gains of 11q23.2-q23.3 were identified in 13/14 samples. Additionally, several other recurrent CNAs were shown,

including gains of 7q34-qter, gains of 12pter-p12.2, gains of 18q21.2, gains of 19pter-p13.2, and losses of 6q14.3-q22.2. The third cohort, including 6 cases with 11q gain/loss determined by array study (two cases from cohort 1 and one case from cohort 2), all the cases were identified to carry 11q gain/loss pattern. Also, losses of 6q (the minimal region was 6q14.1-21) were seen shown in four cases. For gene expression results, they proposed that several genes located in the minimal region of gain at 11q23.3, including *PAFAH1B2*, *ZNF259*, *PCSK7*, *CEP164*, *UBE4A*, and *ATP5L*, were significantly overexpressed, and several genes located in the minimal region of loss at 11q24.1-qter, including *FLI1*, *SNX19*, *NCAPD3*, and *ACAD8*, showed significantly lower mRNA expression in those patients with 11q gain/loss pattern (21).

In 2018, the Grygalewicz group presented 11 male cases with an age between 20-62 years old and two main types of rearrangement were seen in their study. The first type, identified in six of the cases, consisted with a duplication of a fragment of 11q (>50Mbp) and a terminal deletion. The duplicated regions ranged from 71.39 Mbp (11q12.1-q24.3) to 51.27 Mbp (11q13.2-q24.1) and the deletion regions ranged from 6.75 Mbp (11q24.3-q25) to 21.09 Mbp (11q23.2-q25). The second type, verified in four of the cases, comprised of a small duplication of 11q (<20 Mbp) with an additional gain inside the duplicated region, and with a terminal deletion. The duplicated regions ranged from 11.95 Mbp (11q22.3-q24.1) to 18.97 Mbp (11q22.2-q23.3), the additional gains covered region of 1.46 Mbp to 5.26 Mbp, and the terminal deletion regions ranged from 11.36 Mbp (11q24.1-q25) to 15.12 Mbp (11q23.3-q25)(20).

In 2019, the Gonzalez-Frarre group reported 11 cases in their study. Seven of the cases had a typical 11q gain/loss pattern, 2 cases with only terminal deletion, 1 case presented two gains and losses, and one case identified with cnLOH of 11q23.3-q25 (18). In addition, the Wagner group proposed a similar result as well with 13 out of 15 cases with typical 11q gain/loss pattern and 2

remaining cases with only a terminal loss confirmed by FISH. Other recurrent imbalances reported included trisomy 12 (7/15) and gains in 7q with losses in 14q concurrently in 3 out of 15 cases (19).

Section 2. Method and Material

Sample selection

Different from previous studies presenting specific findings in patients classed as BLL with 11q aberrations, our study aimed to broadly evaluate the frequency of 11q aberrations in different large B cell lymphoma subtypes, either Burkitt lymphoma (all ages), diffuse large B cell lymphoma/high grade B cell lymphoma (CD10+ or BCL6+, BCL2-, "Burkitt-like"), and diffuse large B cell lymphoma (germinal center subtype, BCL2+). All of the patients were identified from use of search terms (i.e., "Burkitt lymphoma", "diffuse large B cell lymphoma") and age criteria from the UW Pathology Powerpath database (IRB: No. 2837, PI Chhieng; IRB: No. 13041, PI Ho and Eckel). Cases identified were from between 2000-2020. Cases were reviewed by two hematopathologists (Dr. Eckel, Dr. Naresh) for review of diagnosis according to current WHO classification. Cases with sufficient FFPE tissue were requested for curls from SCCA Laboratories/Fred Hutch or from UW NWBiotrust. Sample types include excision, punch, and biopsy, incision, needle cores, and resection. Sample sites vary in patients. Additional details including age, gender, sample description, immunohistochemical expressions, and cytogenetic studies are summarized in **Table 4**.

Immunohistochemical studies

Immunohistochemical studies were performed part of routine clinical workup in our institution. In brief, stains of primary focus included CD10, BCL6, MUM1, BCL2, CYCLIND1, MYC, and Ki-67.

DNA/RNA extraction and genomic array

AllPrep DNA/RNA FFPE Kit was used for DNA/RNA extraction in our study. DNA and RNA were released sequentially by differential solubilization and treated separately to remove formaldehyde cross-links as well as purified. Purification was performed by QIAcube instruments. Prior to purification, samples underwent deparaffinization via deparaffinization solution (cat. No. 19093) allowing for the exposure to proteinase K. For genomic array, Infinium CytoSNP-850K assay was used and a total of 200ng genomic DNA was used according to manufacturer's protocol. DNA quantitation was using AccuBlue Broad Range dsDNA Quantitation Kit, image of BeadChip was read by iScan Reader, and the array analysis was performed by Nexus Copy Number software (version 10.0).

Data organization

R software was used to summarize our data.

Section 3. Results

Summary of patient selection and quality/quantity of cases at this point

Patient group 1: Patients of all ages diagnosed with BL (n=10)

Patients diagnosed by pathology diagnosis with BL in our institution were included in this group. We included patients of all ages to allow for sufficient cases as the prevalence of Burkitt lymphoma is relatively low in the US. Sixty-two patients were identified from the search of Powerpath data. Of these patients, 25 cases were requested from SCCA/NWBiotrust (after review of case and estimation of remaining tissue), and 16 cases were received for array study (after second review of cases by SCCA/NWBiotrust). The median age of patients with array data in this group is 41.

All of the cases had MYC rearrangement according to FISH study or karyotype, and four of these cases had extra signals of IGH.

Patient group 2: Patients under 60 years old diagnosed with DLBCL or HGBCL with CD10 or BCL6 positive and BCL2 negative (n=5)

This group including patient diagnosed with DLBCL or HGBCL with CD10 or BCL6 positive, and BCL2 negative, under age 60 years old. We excluded patients with double-hit lymphoma. Twenty-eight patients for this group were identified from the search of Powerpath data. Of these cases, 15 cases for this group were requested from SCCA/NWBiotrust, and 10 cases were received for array study. The median age of patients is 42.5. In this group, all but one of the patients lacked MYC rearrangement confirmed by FISH study or karyotype, which defined as "Burkitt-like" group. One of the patients has already been diagnosed with BLL-11q by send-out FISH study, although initial genomic array had not been of sufficient quality for clinical reporting.

Patient group 3: Patients under 60 years old diagnosed with DLBCL or HGBCL with CD10 or BCL6 positive and BCL2 positive (n=4)

Thirteen cases for this group were requested from SCCA/NWBiotrust, and 9 cases were received for array study. The median age of the patients is 52. None of the patients in this group had MYC rearrangement and two of them had extra copy of MYC confirmed by FISH study or karyotype. One case, case 7, had a follicular lymphoma history.

In total, 35 cases had been received from archive, and, at this time point, we have 23 patients with available array data, and 19 patients with sufficient quality score to report (quality score of each sample is summarized in **Table 4**).

The quality score of the samples varied in our study (higher score is worse quality). We found that the sample age showed correlation with the quality score ($R^2 = 0.548$) (**Figure 3)**. We chose to define cases at this point in our study with a quality score over 1.0 as insufficient quality to be reported, leading to 19 remaining cases, 10 cases in group 1 (BL), 5 cases in group 2 (Burkitt-like), 4 cases in group 3 (DLBCL with positive BCL2), at the time point of this writing.

Immunophenotypic and morphologic characteristics of the three groups

Group 1, the Burkitt lymphoma group, consisted of 7 male patients and 3 female patients. Five of the patients were identified to have HIV infection. In accordance of the diagnostic criteria for BL, Ki-67 was high (>95%) in most of the patients (9/10), and CD10 was positive in 10 of the patients. BCL2 was negative in 8 and weak in 2, BCL6 was positive in 5, variable in 2 and not done in 3, MUM1 was rarely performed and varied in 3 cases. All cases showed a *MYC* rearrangement; in two cases the rearrangements were not (8;14), and four patients were specifically noted to have extra copy of IGH. EBER was done in 2 of the cases and was negative in both.

Group 2, our "Burkitt-like" group, consisted of 3 male and 2 female patients. Most patients had a Ki-67 range from 50% to 95%, with one case greater than 95%. CD10 was found positive in all 5 of the patients; it was noted to be dim in one case, BCL2 is negative in 5, BCL6 was positive in all 5, MUM1 was variable in two, negative in two, and not done in one. One case showed a *MYC* rearrangement. EBER was done in 3 cases and was reported as negative in 2 and rare positive cells in 1.

Group 3, patients with DLBCL and positive BCL2 less than 60 years old, consisted of 3 male and 1 female patient. Ki-67 were variable from range 40-95% in this group of patients, CD10 was positive in 3 and negative in 1, BCL6 was positive in all 4, BCL2 is positive in 3 and weak in 1, MUM1 was negative in 3, and not done in 1. No case showed a *MYC* rearrangement; two patients were specifically noted to have extra copy of *MYC*. EBER was done in 3 cases and negative in all 3. A summation of the IHC results (shown individually in **Table 4**) is provided in **Table 5**.

11q aberration and overall array findings

The overall quantifications of alterations identified by genomic array for each group are summarized in **Table 6**. In total from our cases 51 gains, 59 losses, and 22 LOH have been identified. On average, 2.7 gains per case (range 1-13), 3.1 losses per case (range 1-9), and 1.2 LOH per case (range 1-11) have been detected. In the first group (BL), 0.6 gains per case (range 0-3), 3.2 losses per case (range 0-9), 1.6 LOH per case (range 0-11), and an average of 4 gain/loss were found per patient. In the second group (BL-like), 4 gains per case (range 0-10), 1.6 losses per case (range 0-5), 1.2 LOH per case (range 0-3), an average of 5.6 gain/loss were identify per case. In the third group, 6.3 gains per case (range 1-13), 4.8 losses per case (range 1-9), and an average of 8.6 gain/loss were shown per group, and no LOH were identified.

No typical 11q gain/loss pattern as defined in the provisional chapter in the WHO was found in our patient cohorts. One patient in the BL group was identified with 11q terminal deletion (chr11:123,636,573-135,086,622/q24.5-q25), which is within the regions identified by prior BLL-11q studies.(18,21) The features of this patient was reviewed and findings were consistent with WHO defined Burkitt lymphoma, including starry-sky pattern, high Ki-67 (95%), CD10 without BCL2, and the MYC t(8;14). In addition, the array study pointed out a gain on chromosome

12p13.33-p12.1, a loss on chromosome 13q13.3-q21.2, and a loss on chromosome 17q25.3 in this case. The copy number change of 11q of this patient was shown in **Figure 4**.

In addition to 11q, other alterations were identified. In group 1 (BL), two recurrent alterations were seen, loss of heterozygosity of chromosome 6p25.3 (30%) and losses of chromosome 17q25.3 (20%). Previously defined copy number aberrations in Burkitt lymphoma (gains of 1q, 7q, losses of 17p, and abnormalities of 13q) were each found once in our data (**Table 7**). In group 2, "Burkitt-like", no CNA was identified in more than one patient in the group. Summary of the CNAs identified in this group are provided in **Table 8**. In group 3 (DLBCL, BCL2+), five recurrent chromosome abnormalities were reported, including losses on chromosome 1p36.33-p36.23 (50%), gains on chromosome 2p16.1-p15 (50%), gains on chromosome 3p26.3-p21.1 (50%), gains on chromosome 21q11.2-22.3 (50%), and gains on chromosome Xp22.33 (75%) (**Table 7).** These results are consistent with findings from a previous study which also reported losses of chromosome 1p31.1-p36.33 (~25%), gains of chromosome 2p13.3-p25.3 (~10%), gains of chromosome 3 (~50%), and gains of chromosome 21q21.1-q23.3 (~20%) in 64 DLBCL patients (22).

Section 4. Discussion

BLL-11q is a provisional entity in the WHO defined as a subset of HGBCL with clinical and morphological features similar to BL but lacking *MYC* rearrangement and demonstrating a common 11q gain/loss pattern. Whether BLL-11q should be a new entity or a variant of other HGBCLs is still controversial. The diagnosis of BLL-11q is difficult due to limited cases reported and poor understanding of clinical, morphological as well as molecular characterizations. With recent improvement in genetic testing, it is of interest to have a more thorough view of genetic profile of these patients to help clinical classification. Therefore, our study sought to evaluate the frequency of 11q and other copy number aberration in our patients and compare their clinical and morphological features.

First, we aimed to answer whether BLL-11q aberration is exclusive to a single category of large B cell lymphomas. Within the patients included in our study, case 11, clinically classified as Burkitt lymphoma, was confirmed to have 11q terminal deletion by array. No other cases from our other 18 patients were identified to have 11q aberrations, including those of our "Burkitt-like group. The groups of patients are currently too limited for meaningful statistical assessment of the frequency of 11q aberrations, but suggest that 11q aberrations are not highly frequent in our "Burkitt-like" cases.

Looking more broadly at total and other copy number aberrations from our array data, our findings thus far do reflect previous studies on patients with BL and DLBCL. Our first group of patients, patients with BL, has the least average CNAs, which is indicated in many prior papers that patients with BL usually shows a lower genomic complexity (23,24). However, the recurrent alterations of BL proposed by other studies were found only once by us (23–25). In addition, according to prior study, the prevalence *MYC* rearrangement in DLBCL is ~12% (26–28). This is

also supported by our study that one of nine DLBCL patient (group 2 and group 3) had been verified with *MYC* translocation (11%).

We have several future directions for this project. First, to run genomic array on remaining received samples and analyze the data to include in our study. Second, to request more recent samples to get samples with better quality score. Third, to consider including patients from Seattle Children's Hospital (additional 83 cases could be considered for inclusion in our study). We also aim to eventually use stored RNA that has been extracted from the samples for transcriptome assessment, depending on RNA quality.

An important observation made by the study, is the limitations on performing genomic array on small needle cores of tissue, and particularly on older FFPE samples. One prior study indicated a decreased of quantity and quality of DNA extracted from FFPE after a storage of 4-6 years by showing a 3.3 (0.7-17.6) fold decrease in library yield and a 4.5 (1-22.8) fold raise in single-nucleotide variant rate caused by cytosine to uracil deamination in the formalin fixation process (29). Another study highlighted the feasibility of using different specimen types by pointing out that NGS testing was successful in 97% of excision samples but only completed in 31% of needle core specimens (30).

Patient#	Diagnosis	Age	Gender	Sample type	Sample Site	Array quality Score	CD10	BCL6	MUM1	BCL2	CYCLIND1	MYC	Ki-67	EBER	Cytogenetic Studies	Other
2	BL	20	M	Excision	Descending colon	0.2333	+	+	-	-	-	+ (variable, 90% of cells)	>95%	-	MYC rearrangement by FISH (NOT 8;14), no rearr. BCL2 or BCL6	
11	BL	28	M	Excision	Liver mass	0.5386	+	+ (variable)	ND	-	ND	ND	80	ND	MYC rearrangement by FISH, extra signal of IGH, prior case showed 8;14	HIV
12	BL	49	M	Biopsy	Posterior maxilla	0.5183	+	+	ND	+ (small subset)	ND	ND	>95%	ND	MYC rearrangement by FISH	
13	BL	44	F	Biopsy	Neck mass	0.5126	+	+ (variable)	+	-	-	+	>98%	ND	MYC rearrangement by FISH, extra signal of IGH (NOT 8;14)	
14	BL	45	F	Biopsy	Parotid	0.3082	+	+	ND	-	ND	ND	>95%	-	MYC rearrangement by FISH, no rearr. BCL2 or BCL6	HIV
19	BL	41	F	Excision	Ovarian tumor/small bowel tumor	0.994	+	ND	ND	-	ND	ND	99-100%	ND	MYC rearrangement (8;14) by karyotype	HIV
20	BL	41	M	Insicion	Neck mass	0.557	+	ND	ND	-	ND	ND	>95%	ND	MYC rearrangement (8;14) by karyotype	HIV
22	BL	24	M	Excision	Axillary lymph node	0.1694	+	+	ND	-	-	ND	>95%	ND	MYC rearrangement (8;14) by FISH, no rearrangmeent BCL2 or BCL6	
23	BL	45	M	Needle cores	Thigh mass	0.275	+	ND	ND	-	ND	ND	100%	ND	MYC rearrangement and extra copy of IGH, no rearrangement BCL2 or BCL6	HIV
24	BL	66	M	Needle cores	Periumbilical mass	0.15	+	+	+ (rare cells)	+ (weak)	-	+ (variable)	95%	ND	MYC rearrangement (8;14) by FISH, no rearrangmeent BCL2 or BCL6	
18	"BL like" (large B, BCL2-)	29	M	Excision	Testis	0.7343	+ (dim)	+	+ (subset)	-	ND	ND	>95%	-	MYC rearrangement (8;14) by FISH	
1	"BL like" (large B, BCL2-)	58	F	Incision	Femur	0.0983	+	+	-	-	-	ND	90%	ND	No rearrangment of MYC by FISH	
3	"BL like" (large B, BCL2-)	46	M	Excision	Neck mass	0.1057	+	+	+ (weak, 20%)	-	ND	ND	90%	+ (rare pos cells)	No rearrangment of MYC by FISH	
8	"BL like" (large B, BCL2-)	38	F	Excision	Lymph node, neck	0.1915	+	+	-	-	- (prior case)	+ (variable, 20-40%) (prior case)	50%	- (prior case)	No rearrangment of MYC by FISH, also no rearrangmeent of BCL2 or BCL6	
25	"BL like" (large B, BCL2-)	39	M	Resection	Groin lymph node	0.337	+	+	ND	-	ND	0.1	75-85%	ND	No MYC rearrangement	
4	DLBCL, BCL2+	51	M	Incisional	Retroperitoneal nodules	0.1261	+	+	-	+	-	ND	40-50%	-	No MYC rearrangement, positive for extra copy of MYC	
5	DLBCL, BCL2+	56	F	Excision	Supraclavicular neck mass	0.1436	+	+	-	+	-	10-20%	90-95%	-	No MYC rearrangmeent (prior case showed t(14;18), neg for BCL6 rearr)	
6	DLBCL, BCL2+	33	M	Excision	Axillary lymph node	0.0795	-	+	-	+ (weak, subset 30-40%)	+ (small subset, variable)	20-25%	40-50%	-	No MYC rearrangement, positive for extra copies of MYC	
7	DLBCL, BCL2+ (with FL)	53	M	Excision	Cervical lymph node	0.1074	+	+	ND	+	-	40%	60%	ND	No MYC rearrangement	

TABLE 4: Clinical characteristics of patients in our groups.

Diagnosis, age, gender, sample types, biopsy sites, array quality score, IHC, cytogenetic studies of ten patients in the BL group, five patients in the BL-Like group, and four patients in the DLBCL, BCL2+ group are shown in table.

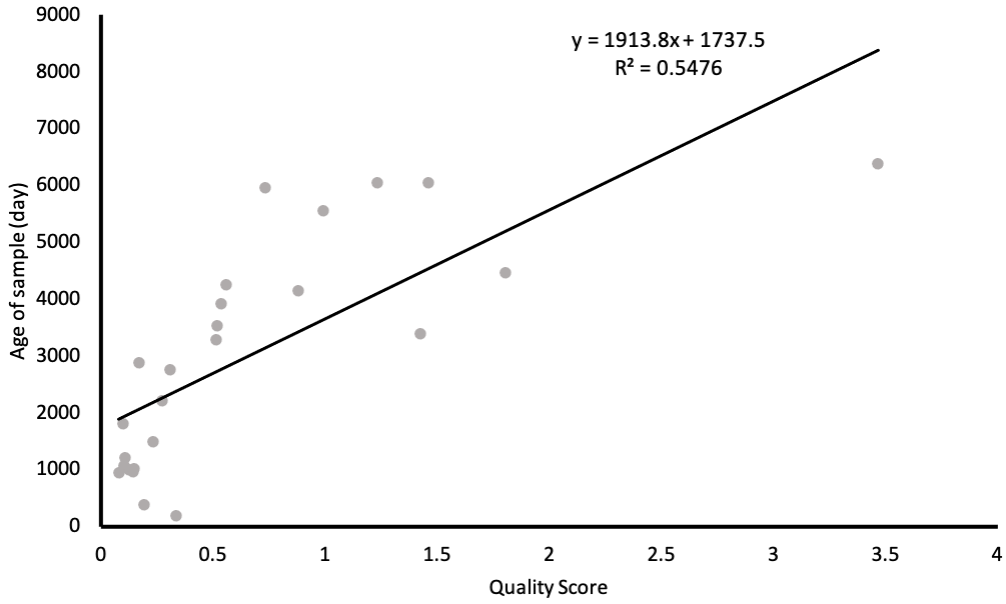

Figure 3. Plot of the correlation of the age of the samples and the corresponding quality score.

	Ki-67	CD10	BCL-2	BCL-6	MUM1	MYC rearrangement
Group 1 (10 Burkitt patients)	>95% in 9	+ in all 10	- in 8 weak + in 2	+ in 5 variable in 2 ND in 3	Rarely performed varied in 3	+ in all; Two of the cases were not t(8;14)
Group 2 (5 patients with DLBCL, positive for BCL2)	Range from 50%-95% in 4, >95% in 1 patients	+ in all 5	- In all 5	+ in all 5	Variable in 2 - in 2 ND in 1	+ in 1
Group 3 (4 patient with DLBCL, positive for BCL2)	Variable from 40%-95%	+ in 3 and - in 1	+ in 3 weak + in 1	+ in all 4	- In 3 ND in 1	- in all

TABLE 5. Summary of IHC results and MYC rearrangement status in each group of patients.

Expression of Ki-67, CD10, BCL-2, BCL-6, and MUM1, and the status of MYC rearrangement in each group of the patients were shown in the table.

Group	Gain	Loss	LOH	Total gain/loss
1 (BL)	0.6 (0-3)	3.2 (0-9)	1.6 (0-11)	4
2 (B-like)	4 (0-10)	1.6 (0-5)	1.2 (0-3)	5.6
3 (DLBCL, BCL2+)	6.3 (1-13)	4.8 (1-9)	0	8.5

Table 6. Mean, range, and total number of abnormalities per group.

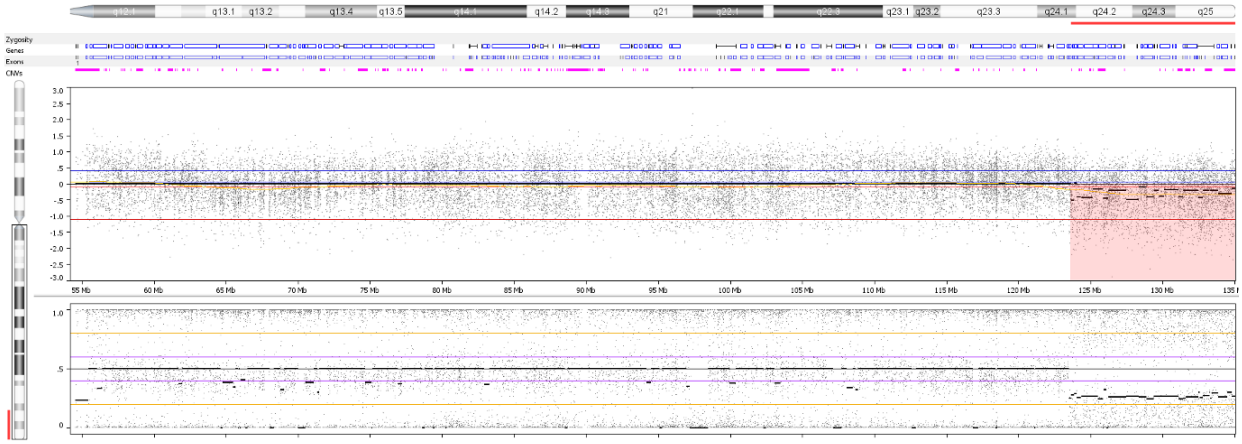

Figure 4. The 11q terminal deletion presented in case 11.

Group	Most common abnormalities	Event	Frequency
1	chr6 p25.3	LOH	0.30
(BL)	chr17 q25.3	CN Loss	0.20
	Chr1 q21.1-q44	*CN Gain*	*0.10*
	Chr13 q31.3	*CN Gain*	*0.10*
	Chr13 q	*CN Loss*	*0.20*
	Chr17 p12	*CN Loss*	*0.10*
	Chr7 q	*CN Gain*	*0.10*
3	chr1 p36.33-p36.23	CN Loss	0.5
(DLBCL, BCL2+)	chr2 p16.1 - p14	CN Gain	0.5
	chr3 p26.3 - p21.1	CN Gain	0.5
	chr21 q11.2 - q22.3	CN Gain	0.5
	chrX p22.3	CN Gain	0.75

Table 7. Summary of copy number alterations other than 11 in group 1 (BL) and group 3 (DLBCL, BCL2+, under 60 years old) patents.

Two recurrent alterations were seen in group 1 including, loss of heterozygosity of chromosome 6p25.3 (30%) and losses of chromosome 17q25.3 (20%). The Common Burkitt lymphoma CNAs were not identified recurrently in our patients, including gains of 1q, 7q, losses of 17p, and abnormalities of 13q. Five recurrent chromosome abnormalities were reported in group 3 and the results are consistent with previous study.

CN Gain	CN Loss
chr1 p31.1	chr3 q27.3 - q29
chr1 q22 - q44	chr4 q31.21
chr1 q44	chr6 p22.3
chr2 p16.1 - p14	chr6 q14.1 - q27
chr3 p14.1 - p13	chr10 q21.1 - q26.3
chr3 p22.2 - p22.1	chr16 p11.2
chr3 q26.1 - q29	chr19 p13.3 - p11
chr4 p16.1	chr19 q13.32
chr4 q13.1	
chr6 p25.3 - p21.1	
chr9 p24.3 - q34.3	
chr11 p15.5 - p12	
chr12 q11 - q14.1	
chr13 p11.1 - q34	
chr16 q24.1	
chr17 p13.1	
chr17 p13.3 - q25.3	
chr19 p13.3 - p13.11	
chr22 p11.1 - q13.33	
chrX p22.33	

Table 8. List of gains and losses found in group 2 (DLBCL, BCL2-) of patients.

Each of the event was verified only once in our study.

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