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ORIGINAL ARTICLE

Incorporation of digital gene expression profiling for cell-of-origin determination (Lymph2Cx testing) into the routine work-up of diffuse large B cell lymphoma

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Abstract

Diffuse large B cell lymphomas (DLBCL) represent a clinically heterogeneous group of lymphomas that are classified together based on similarities in morphology and immunophenotype. Gene expression profiling further classifies DLBCL into distinct molecular subgroups based on cell-of-origin (COO), including germinal center B cell type, activated B cell type, and unclassified type. COO assignment of DLBCL has important biological and prognostic significance, as well as emerging therapeutic implications. Herein, we describe the first clinical validation of a digital gene expression-profiling assay (Lymph2Cx) to perform COO assignment in the routine work-up of DLBCL using formalin-fixed paraffin-embedded (FFPE) tissue sections and describe the results of 90 consecutive DLBCL cases analyzed prospectively by a College of American Pathologists/Clinical Laboratory Improvement Amendments (CAP/CLIA)-certified clinical molecular diagnostics laboratory.

Keywords Diffuse large B cell lymphoma · Cell-of-origin · Lymph2Cx · Gene expression profiling

Introduction

Diffuse large B cell lymphoma (DLBCL) is the most common type of non-Hodgkin lymphoma, comprising approximately 25–30% of adult non-Hodgkin lymphomas in western countries, and consist of a clinically heterogeneous group that exhibits similarities in morphology and immunophenotype [1, 2]. However, it was found that gene expression profiling (GEP) could further classify DLBCLs into distinct molecular subgroups based on cell-of-origin (COO), including the germinal center B cell type (GCB), activated B cell type (ABC), or unclassified (UNC) type, and that these subtypes had important prognostic significance, such that patients with ABC-DLBCL exhibited a significantly worse outcome when treated with R-CHOP (rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone) chemotherapy [3–5]. However, the original classification method required the use of fresh or fresh-frozen tissue for GEP COO assignment using the Affymetrix microarray platform (Santa Clara, CA) and was not practical for everyday clinical use. This led to the development of simpler and less expensive immunohistochemistry (IHC)-based methods for assignment of COO using FFPE tissue sections, including the initial algorithm developed by Hans et al [6]. Although several additional IHC schemes were subsequently developed, IHC assignment of DLBCL COO continues to exhibit poor reproducibility and agreement with the gold standard GEP assay. A recent inter-institutional comparison of nine IHC algorithms for DLBCL COO classification reported poor concordance across the different algorithms, with only 4.1% concordance of the tumors classified as GCB and 21% as ABC/non-GCB by all IHC methods [7]. In order to make molecular COO assignment of DLBCLs more practical, accurate, and reproducible, a digital GEP assay was developed for the nCounter platform (NanoString, Seattle, WA) using FFPE tissues, known as the

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"Lymph2Cx" assay [8]. This assay uses a 20-gene panel selected for the ability to accurately replicate the COO model of Lenz et al. [4], and includes 8 genes overexpressed in ABC-DLBCL, 7 genes overexpressed in GCB-DLBCL, and 5 housekeeping genes. This assay demonstrated more than 95% concordance of COO assignment when compared with the original frozen tissue based microarray classification, with very few failed cases, and also retained prognostic significance, making it a viable alternative for testing in a conventional clinical molecular diagnostic laboratory setting. Here, we describe the first clinical application of the Lymph2Cx assay in a CAP/CLIA-certified molecular diagnostics laboratory, describe incorporation of the assay into the routine work-up of DLBCL cases, and summarize the results of the first reported Lymph2Cx assay clinical case series.

Materials and methods

Specimens

Twenty DLBCL FFPE tissue specimens previously characterized for COO by Affymetrix microarray GEP on matched fresh/frozen tissue, 1 DLBCL cell line each from GCB-DLBCL (DB) and ABC-DLBCL (Riva) subtypes, and 11 additional DLBCL FFPE tissue specimens (excisional and needle core biopsies) obtained from Mayo Clinic Arizona and Mayo Clinic Rochester archives were analyzed as a part of the Lymph2Cx clinical assay validation. All 90 sequential samples analyzed by the Molecular Diagnostics–Arizona Laboratory (MDAZL) at Mayo Clinic Arizona from December 2016 to December 2017 were included in the study, and included 59 excisional biopsies, 30 needle core biopsies, and 1 pleural fluid cellblock. During this time frame, there were 8 additional cases submitted for testing that were not able to be analyzed, including 5 due to insufficient tissue, 2 due to insufficient tumor, and 1 with poor quality RNA.

Lymph2Cx assay

To facilitate pathology review, H&E-stained slides were subjected to whole slide scanning using an Aperio ScanScope AT Turbo (Leica Biosystems, Inc.; Buffalos Grove, IL) digital whole slide scanner. Internet-based image viewing was utilized to confirm the presence of tumor tissue and to estimate tumor content as a percentage of tissue area by board-certified hematopathologists (R. S. R. and L. M. R.) using Aperio Spectrum Imagescope software. This obviated any need for transfer of slides to and from the MDAZL lab and hematopathology hospital sign out areas. All annotation functions, such as circling areas of the slide for tissue macrodissection were performed using the Aperio system software tools. Tumor tissue comprising ≥ 60% of the surface area was macrodissected from corresponding 5 or 10 μm thickness unstained FFPE tissue sections to remove non-tumorous tissue. The number of unstained tissue sections used for the assay varied from 1 to 5 based on total tissue size and section thickness to yield approximately 1cm^2 tumor tissue area. Total RNA was extracted using High Pure FFPET RNA Isolation Kits (Roche; Product No. 06650775001), and the resulting RNA was quantified by spectrophotometry (NanoDrop™, Thermo Fisher Scientific, Waltham, MA). Four hundred ng total RNA was hybridized overnight on a thermal cycler (FlexCycler; Analytik Jena US, Inc., Upland, CA) to the 20 fluorescently labeled gene probes in the Lymph2Cx panel at 67 °C for 16 h. The 20 gene panel includes 8 genes overexpressed in ABC-DLBCL (TNFRSF13B, LIMD1, IRF4, CREB3L2, PIM2, CYB5R2, RAB7L1, CCDC50), 7 genes overexpressed in GCB-DLBCL (MME, SERPINA9, ASB13, MAML3, ITPKB, MYBL1, S1PR2), and 5 housekeeping genes (R3HDM1, WDR55, ISY1, UBXN4, TRIM56) [7]. Probe/RNA complexes were purified on a NanoString® nCounter® Prep Station (NanoString Technologies, Inc., Seattle, WA) to remove excess probes and then bound, immobilized, and aligned on a nCounter® Cartridge for analysis. Up to 12 samples can be processed in a single nCounter® Cartridge, but 2 wells on each cartridge were reserved for positive (GCB-DLBCL sample) and negative (nuclease-free water) control samples. Sample cartridges were placed on a NanoString nCounter® Digital Analyzer for quantification via single-molecule imaging. Quality control (QC) analysis was performed for each sample cartridge using NanoString nSolver™ Analysis Software to check the 4 standard nCounter-specific quality control parameters, including imaging QC, mean binding density QC, positive control linearity QC, and positive control limit of detection QC. The counts were processed using the National Cancer Institute's Lymphoma/Leukemia Molecular Profiling Project Lymph2Cx DLBCL COO Classifier (patented algorithm, available through National Cancer Institute website via Data Use Agreement) [8]. The assay produces a calculated score on a scale of 0.00 to 1.00 to classify each DLBCL sample based on the probability that the sample is ABC type [8, 9]. Samples with a ≥ 90% probability of being ABC-DLBCL type (a score of 0.90–1.00) are classified as ABC-DLBCL, samples that have a ≥ 90% probability of being GCB-DLBCL type (a score of 0.00–0.10) are classified as GCB-DLBCL, and samples with scores > 0.10 and < 0.90 are categorized as "Unclassifiable" (UNC-DLBCL).

Case characterization

IHC subtype assignment of DLBCLs (GCB versus non-GCB) was also made for 77 of the total 90 clinical DLBCL cases using the Hans algorithm, with stains for CD10, BCL6, and

MUM1 per local practice [6]. The results from the original pathology reports were recorded for correlation with the Lymph2Cx results [10–12]. Similarly, 89 of the total 90 DLBCL clinical samples had been clinically evaluated for rearrangements of the *MYC*, *BCL2*, and *BCL6* loci using conventional FISH techniques with Vysis LSI Dual Color Break Apart (BAP) Rearrangement Probes for the *MYC*, *BCL2*, and *BCL6* genes obtained from Abbott Molecular, Inc. (Des Plaines, IL).

Statistical analysis

Distributions and contingency analysis were created using JMP statistical software (SAS Institute Inc., Cary, NC). All *p* values were calculated using the likelihood-ratio chi-square method. *P* values < 0.05 were considered to be statistically significant.

Institutional review board review

This study was performed with Mayo Clinic Arizona Institutional Review Board approval under protocol #17-006519.

Results

Assay Performance

Lymph2Cx COO testing of 20 FFPE specimens (12 ABC cases and eight GCB cases) that had previously been characterized for COO by Affymetrix microarray GEP in the original Lymph2Cx COO assay validation sample cohort [8] on matched fresh/frozen tissue, and one DLBCL cell line each from GCB (DB) and ABC (Riva) subtypes, showed 100% concordance with previous COO testing results. Performance characteristics for the NanoString® nCounter® system and the Lymph2Cx COO GEP assay were also established. The lower level of RNA input was established at 400 ng. Unstained sections stored up to 2 weeks were suitable for use resulting in the same COO calls in 5 cases. The minimum laboratory time required for the analysis was 28 h, with a median time of 2.4 business days (range 1–5 days). Nine of the 11 additional DLBCL FFPE validation specimens (excisional and needle core biopsies) obtained from Mayo Clinic archives also showed successful COO designation, with 6 GCB cases, 2 ABC cases, and 1 UNC case identified. Two of the validation samples could not be analyzed: one due to insufficient RNA quantity (that still produced a result when processed with the assay) and one that failed assay quality metrics (indicated degraded RNA). In the first year after the initial laboratory validation of the Lymph2Cx COO clinical assay, our CAP/CLIA-certified molecular diagnostics laboratory successfully analyzed 90 specimens obtained from a wide variety of anatomic sites, and even included 1 pleural fluid cellblock (Supplementary Table 1). These cases included 59 excisional biopsies (65.6%), 30 needle core biopsies (33.3%), and one cellblock (1.1%). On average for all cases, Lymph2Cx testing required 2.3 unstained tissue sections at 10 μm thickness (range 1–6 sections). For excisional biopsies, the average number of unstained sections required for testing was 1.7 (range 1–5 sections), and 3.5 unstained sections (range 1–6 sections) for needle core biopsies. Four of the 90 clinical cases used 4 μm or 5 μm thickness previously cut unstained slides. In these cases, approximately twice the numbers of unstained sections were needed for the extraction process in order to obtain the required 400 ng of RNA. However, all 4 of these cases were successfully analyzed.

Cell-of-Origin

The Lymph2Cx assay detected 42 GCB (46.7%), 34 ABC (37.8%), and 14 UNC (15.5%) cases (Table 1 and Supplementary Table 1). There were 63 male (70%) and 27 female (30%) patients, with a median age of 66 years (range 33 to 96 years). In these predominantly male patients (M F = 2.33), distribution analysis showed no statistically significant associations between patient sex and DLBCL COO subtype (males, $p < 0.8148$; females, $p < 0.64$). This case series included 34 nodal (37.8%) and 56 extra-nodal (62.2%) lymphomas. Only DLBCL, NOS cases were included in the study and the case series did not include any other specific DLBCL subtypes or variants. The extra-nodal lymphomas included a wide variety of tissues and anatomic sites (see Supplementary Table 1). Contingency analysis was performed to assess the relationship between anatomic location (nodal versus extra-nodal) and COO subtype. At 95% confidence, the null hypothesis that there is no relationship between DLBCL COO subtype call and nodal status could not be rejected for the distribution as a whole ($p < 0.36$). However, ABC-DLBCL cases were more than twice as likely to be extra-nodal in both male and female patients (Table 1), and this relationship was statistically significant ($p < 0.015$). Interestingly, the majority of the 14 total UNC cases (9/14; 64.3%) were comprised of extra-nodal lymphomas; however, both GCB-DLBCL and UNC-DLBCL cases failed to show any statistically significant relationship with nodal status ($p < 0.54$ and $p < 0.28$, respectively; Table 1).

Immunophenotype

Seventy-seven of the 90 total cases (85.5%) were also analyzed by immunohistochemistry using the Hans algorithm, and included 48 GCB-DLBCL (62.3%) and 29 non-GCB-DLBCL (37.7%) cases. Among these 77 cases, the overall concordance rate was 79% if all three possible Lymph2Cx

Table 1 Summary of Lymph2Cx cell-of-origin assignment of 90 clinical DLBCL cases and associated findings

Lymph2Cx COO assignment	Sex	Nodal	Extra nodal	MYC/BCL2 GR	MYC/BCL6 GR	MYC/BCL2/BCL6 GR
GCB $n = 42$ (46.7%)		19 (45.2%)	23 (54.8%; $p < 0.5368$)	2 (4.7%)	0	3 (7.1%)
	Male 30 (71.4%)	13 (68.4%)	17 (73.9%)	2 (4.7%) Extra nodal	0	2 (4.7%) Extra nodal
	Female 12 (28.6%)	6 (31.6%)	6 (26.1%)	0	0	1 (2.4%) Nodal
ABC $n = 34$ (37.8%)		10 (29.4%)	24 (70.6%; $p < 0.0148$)	1 (2.9%)	1 (2.9%)	0
	Male 25 (73.5%)	7 (70%)	18 (75%)	1 (2.9%) Extra nodal	1 (2.9%) Nodal	0
	Female 9 (26.5%)	3 (30%)	6 (25%)	0	0	0
UNC $n = 14$ (15.5%)		5 (35.7%)	9 (64.3%; $p < 0.2817$)	0	0	0
	Male 8 (57.1%)	3 (60%)	5 (55.6%)	0	0	0
	Female 6 (42.9%)	2 (40%)	4 (44.4%)	0	0	0

GR gene rearrangements

categories were included in the comparison (i.e., Lymph2Cx UNC-DLBCL classified cases were also considered discordant, since the Hans immunohistochemistry algorithm is binary and does not distinguish this category). If the UNC-DLBCL samples are excluded, the concurrence rate between the Lymph2Cx and Hans algorithm was 97.6% for 41 GCB-DLBCL samples and 91.7% for 24 non-GCB-DLBCL samples, with 2 cases classified as ABC-DLBCL by Lymph2Cx analysis determined to be GCB-DLBCL by the Hans algorithm, and 1 case classified as GCB-DLBCL by Lymph2Cx analysis determined to be non-GCB-DLBCL by the Hans algorithm (see Table 2). Interestingly, 11 of 11 cases that also underwent flow cytometry and demonstrated expression (per clinical pathology report) for CD10 by that method were also classified by Lymph2Cx as GCB-DLBCL subtype.

FISH

Eighty-nine cases were also analyzed by FISH for the detection of possible *BCL2*, *BCL6*, and *MYC* gene rearrangements. A total of 7 cases of high-grade B cell lymphoma, with MYC and BCL2 and/or BCL6 rearrangements were identified (7.8%), and included 4 cases so-called genetic "double-hit" type rearrangements with rearrangements of the *MYC* and *BCL2* and/or *BCL6* genes, and 3 "triple-hit" cases with rearrangements of all 3 genes (Table 1). Lymph2Cx COO testing of these 7 cases of high-grade B cell lymphoma, with *MYC* and *BCL2* and/or *BCL6* rearrangements detected 5 GCB type and 2 ABC type lymphomas. The high-grade GCB type lymphomas consisted of 2 cases with *MYC/BCL2* rearrangements and 3 triple-hit cases with *MYC/BCL2/BCL6* rearrangements, with 4 of the 5 cases occurring at extra-nodal sites. The 2 high-grade ABC type lymphomas included 1 extra-nodal case with *MYC/BCL2* rearrangements and 1 nodal case with *MYC/BCL6* rearrangements.

Discussion

Herein, we report the first laboratory-developed digital GEP assay using NanoString technology available for determination of COO assignment of DLBCLs in a CAP/CLIA-certified clinical molecular diagnostics laboratory. FFPE tissue samples from a variety of sources and biopsy types, including excisional and needle core biopsies of lymph nodes and extra-nodal tissues as well as a cellblock,

Table 2 Comparison of Lymph2Cx GEP-based COO analysis and immunohistochemistry-based COO analysis using the Hans algorithm

Lymph2Cx molecular COO classification results (true GCB and ABC cases)	Hans algorithm (GCB and non-GCB)
True GCB % ($n = 41$)	Concordant 97.6% ($n = 40$)
	Discordant 2.4% ($n = 1$)
True ABC % ($n = 24$)	Concordant 91.7% ($n = 22$)
	Discordant 8.3% ($n = 2$)

yielded sufficient quantity and quality RNA for accurate COO assignment of DLBCLs using the Lymph2Cx assay. Cases were successfully analyzed using either 10 μm thick unstained sections specifically cut for the Lymph2Cx assay (as described in the original publication) or excess 4–5 μm sections left over from clinical purposes and stored for up to 2 weeks. A test validation using unstained sections stored for longer periods of time is currently underway. Use of the Aperio scanning for quick transmission of images between the MDAZL laboratory and molecular hematopathologists has proven to be particularly time efficient. Subsequently, all hematopathologists at the Mayo Conic in Arizona have been trained to annotate their own cases for subsequent Lymph2Cx testing. Lymph2Cx assay results are often available before FISH studies are available, and therefore, do not cause a delay in final diagnosis. In addition, we have noted that a majority of UNC-DLBCL cases originated from extra-nodal sites, and that ABC cases were greater than 2 times more common at extra-nodal sites compared to nodal cases in both male and female patients. We also describe for the first time that CD10 expression by flow cytometry, similar to CD10 by IHC, indicates GCB-DLBCL type as determined with molecular methods. Additional cases will need to be analyzed to determine if these trends persist in a larger case series.

Our current local workflow is illustrated in Fig. 1. When a diagnosis of DLBCL is suspected, every attempt is made to maximize the amount of tissue available for additional downstream testing. In order to maximize tissue available for downstream testing, our local laboratory recently instituted a change in grossing procedure to limit core biopsies to no more than 2 biopsies per tissue cassette so that limited tissue is not as easily exhausted by multiple downstream tests. Immunophenotyping as needed to confirm the diagnosis is first performed. Next, FISH analysis is prioritized to rule out the possibility of high-grade B cell lymphoma, with *MYC* and *BCL2* and/or *BCL6* rearrangements (previously known as genetic "double-hit" or "triple-hit" DLBCLs). FISH is performed in all cases since, although various recommendations for screening strategies have been made, morphology and immunophenotype cannot always predict the presence of high-grade lymphoma translocation patterns with complete accuracy [13]. Per current World Health Organization guidelines for the classification of DLBCL, COO subtyping should be made for all DLBCL cases at diagnosis [1]. If GEP technology is not available, then IHC is considered an acceptable alternative. In this first prospective clinical case series, we demonstrate that the GEP-based Lymph2Cx analysis is a robust, practical, rapid, highly accurate, and reproducible assay that can accurately determine COO in DLBCL.

Our case series also included seven cases of high-grade B cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangements, with five GCB and two ABC type cases identified by Lymph2Cx testing. The two ABC double hit cases included one case with *MYC* and *BCL6* rearrangements and one with *MYC* and *BCL2* rearrangements. To our knowledge, this is the first time the combination of *MYC* and *BCL2* rearrangements have been reported in the literature for ABC type high-grade B cell lymphoma. Prior reports using the Lymph2Cx assay to assign COO have found that *MYC/BCL2* double hit cases occurred exclusively in GCB cases [9, 14, 15]. However, our case series suggests that ABC type double hit cases with *MYC/BCL2* rearrangements do occur, but in the context of current literature findings, appear to be quite rare.

There is mounting clinical evidence that novel therapeutic agents may exhibit selective activity in patients with ABC type DLBCL. For example, a recent phase II clinical trial has demonstrated that R-CHOP combined with lenalidomide (R2CHOP) can overcome the negative prognostic impact of non-GCB-DLBCL [16]. Another recent phase II/III multicenter randomized clinical trial also demonstrated a preferential benefit in non-GCB type DLBCLs that were treated with lenalidomide monotherapy [17]. Furthermore, this benefit was much more pronounced in DLBCLs that were defined as ABC-DLBCL type using GEP subtyping methods rather than immunohistochemistry-based subtyping. Additional studies suggest that accurate COO assays may help to identify patients that may benefit from agents targeting specific COO subgroups [8, 9, 14–20]. Recently, the genetic landscape of UNC-DLBCL was described, making this an important category to identify rather than included as non-GCB-DLBCL as typically done with IHC [21]. Thus, it is becoming more important to perform accurate and reproducible molecular COO determination. Indeed, a number of other groups have also developed molecular methods to determine COO in DLBCL. For example, Mareschal et al. developed a gene-expression assay for COO determination in DLBCL based on a reverse transcriptase-multiplex ligation-dependent probe amplification (RT-MLPA) and also recently expanded their assay to include the classification of primary mediastinal large B cell lymphoma [22, 23]. In addition, Collie et al. developed a novel multiplex single-tube gene expression assay on the ICEPlex® system which could differentiate between GCB and ABC DLBCL subtypes in FFPE tissue specimens [24]. More recently, next-generation sequencing technology has also been utilized for COO determination of DLBCL, with a recent paper from Reddy et al. describing the use of RNAseq for reliable classification of DLBCL [25].

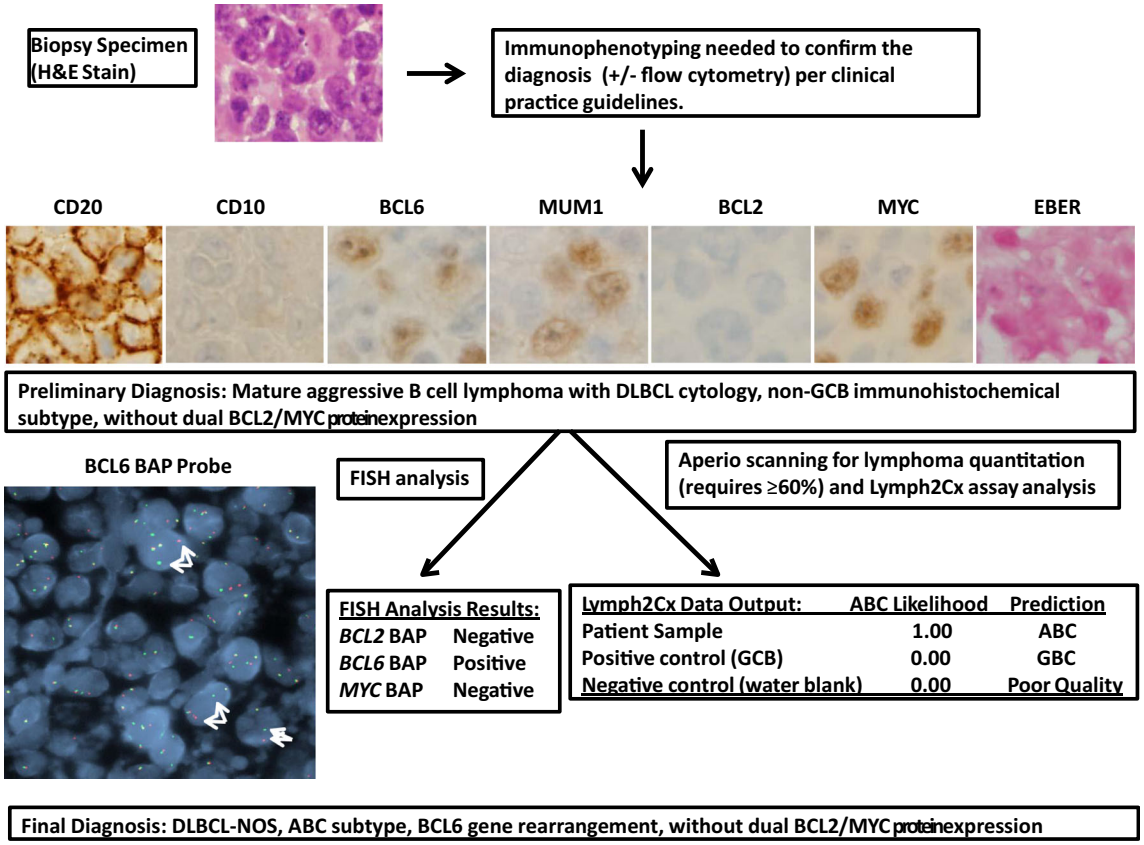

Fig. 1 Example case illustrating proposed tentative workflow for DLBCL diagnosis with incorporation of Lymph2Cx COO assay. When a diagnosis of DLBCL is suspected, excisional biopsies are recommended if possible. However, if core biopsies are obtained, and to facilitate spare use of the tissue, the cores are divided into more than one tissue cassette with no more than 2 cores per cassette. Derivative blocks can also be made after initial H&E assessment to divide cores into more than one daughter block if additional studies are needed for non-lymphoma cases. Initially, sufficient immunophenotyping is performed to confirm the diagnosis based on the amount of available tissue and may include the Hans algorithm immunostains (CD10, BCL6, MUM1), along with additional stains for BCL2 and MYC to identify possible dual expresser lymphomas. If there is sufficient fresh tissue available, flow cytometry may also be performed to aid in establishing the diagnosis and subsequent stains IHC per clinical practice guidelines. After a preliminary diagnosis of large B cell lymphoma with DLBCL or high-grade morphology, unstained FFPE slides are subjected to FISH analysis using break apart probes for the *BCL2*, *BCL6*, and *MYC* genes in order to identify possible cases of high-grade B cell lymphoma, with MYC and BCL2 and/or BCL6 rearrangements. There is usually sufficient tissue remaining after these studies to perform Lymph2Cx COO testing, with a minimum requirement of 60% or more lymphoma present in the specimen in order to perform the analysis. H&E-stained slides are scanned using an Aperio digital whole slide scanner, and the slides are annotated for tumor content using internet-based image viewing and annotation software. Corresponding unstained slides are macrodissected, and the Lymph2Cx COO GEP assay is performed using RNA isolated from the specimen. The figure illustrates an example DLBCL specimen that exhibited a non-GCB type immunohistochemical stain profile using the Hans algorithm. FISH analysis performed on this case identified a *BCL6* gene rearrangement, with no evidence of *BCL2* or *MYC* rearrangements, and Lymph2Cx analysis results were consistent with ABC type COO

Interestingly, although GEP-based testing is considered the gold standard for COO determination in DLBCL, when all of the UNC-DLBCL samples are excluded from the comparison, we found that the concurrence rate between the Lymph2Cx and Hans algorithm within our single institution study showed good correlation, with only 2.4% discrepant GCB cases and 8.3% non-GCB cases (Table 2). A recent study by Reinke et al. also noted that the Hans immunohistochemical algorithm for DLBCL COO determination performed relatively well in an interlaboratory variability study, with CD10 performing as the most reliable IHC marker in terms of reproducibility between laboratories [26]. In summary, this report describes the prospective application of the Lymph2Cx COO assay as a laboratory-developed test in a clinical diagnostics laboratory, includes a number of initial case observations, and also clearly illustrates how this assay can be incorporated into the routine workflow for the workup of DLBCL cases.

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Authors' contributions R.S.R. and L.M.R.: conception, design, data analysis and interpretation, and manuscript editing

C.A.R.: assay design and performance, statistical calculations, and manuscript editing

A.C.R.: case identification and submission and manuscript editing

T.K.Y.: assay performance and manuscript editing

A.J.W.S., B.J.G-G.: assay design and performance and manuscript editing

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Compliance with ethical standards

This study was performed with Mayo Clinic Arizona Institutional Review Board approval under protocol #17-006519.

Conflicts of interest MDAZL: Center for Individualized Medicine, Mayo Clinic.

L.M.R. is a co-inventor on a patent for the NanoString technology used in this manuscript but has received no licensing fees or royalty payments.

Other authors declare that they have no conflict of interest.

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