Optimal Minimal Panels of Immunohistochemistry for Diagnosis of B-Cell Lymphoma for Application in Countries With Limited Resources and for Triaging Cases Before Referral to Specialist Centers

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Key Words: Lymphoma; B-cell lymphoma; Immunohistochemistry; Diagnosis; Classification; Developing countries


ABSTRACT

Objectives: Establish and validate optimal minimal immunohistochemistry panels for usage in a staged algorithmic manner for precise diagnosis of B-cell lymphomas in countries with limited resources. Suggest short panels of immunostains to be used in referring units that refer suspected lymphomas to specialist diagnostic centers in resourceful countries.

Methods: Significant proportion of six B-cell lymphomas has characteristic morphology requiring a short panel of confirmatory immunostains. The rest would go through five different algorithms.

Results: 812 cases in which a B-cell lymphoma or an HIV-associated lymphoma was suspected on morphological grounds were evaluated. This led to arriving at a specific diagnosis of 799 B-cell lymphomas. A correct diagnosis was achievable in 69% cases with the application of three to five antibodies; others required additional work-up.

Conclusions: The panels/algorithms assist pathologists in practicing lymphoma diagnostics in countries with limited resources and in making lymphoma referrals to specialist centers.

Lymphomas are a collection of different malignancies “arising” from lymphoid cells. They include about 49 entities, and over 19 provisional entities and subsets.1 About 85% of lymphomas are of B-cell origin. Precision in lymphoma diagnosis requires expertise and infrastructure. The entities are defined based on morphology, immunohistochemistry (on some occasions in situ hybridization), cytogenetics/fluorescent in situ hybridization (FISH), molecular genetics and clinical information. Thorough knowledge and experience in morphology of lymphoid lesions and a good understanding of the current World Health Organization (WHO) classification of lymphoid neoplasms are essential.1 Accurate diagnosis also requires application of a wide panel of immunohistochemical stains, high-quality laboratory infrastructure and personnel, and resources. In addition, about 20% of cases need cytogenetic and molecular investigations.

Though the incidence of lymphomas varies widely across the globe, and though the incidence is higher in countries with better resources, lymphomas are frequent in countries with limited resources.2 As many countries with limited resources have high population density, and as the numbers of hematopathologists are unfortunately low, the absolute numbers of lymphomas seen by individual pathologists are considerably high. Practicing lymphoma pathology in developing countries is challenging, and two of the authors (K.N.N. and L.L.) have been amply exposed to these challenges.3-7

In many countries within Europe, lymphoma diagnosis is being centralized to specialist-integrated hematologic...
malignancy diagnostic services (SIHMDS). Within many SIHMDS networks, pathologists at contributing/referring units would like to maintain their expertise. Pathologists from the referring units would prefer to perform some immunostains, attempt to arrive at a diagnosis, and then forward the sample to the SIHMDS for final integrated diagnosis. Although this helps in maintaining a certain level of expertise at referring units, it also results in duplication of work-up and a strain on resources.

Though lymphoma diagnostics have evolved in resourceful countries of Europe and North America, some of the investigators in these countries (such as two of the authors of this manuscript, L.L. and K.N.N.) have a keen interest in improving lymphoma diagnostics in developing countries and in fostering translational research in lymphomas prevalent in developing countries. The primary aim of the study was to establish minimal panels of immunohistochemistry that can be used in a staged algorithmic manner to arrive at a precise diagnosis in most cases of suspected B-cell lymphomas in countries with limited resources. Through this work, we also wanted to suggest short panels of immunostains that can be used in referring units in the context of SIHMDS networks (or similar centralized services for lymphoma diagnostics) before the referral is made. The limited panel algorithms are not intended for use in SIHMDS, where having short turnaround times (TATs), achieving highest accuracy and diagnostic precision on a large volume of cases, training of personnel within the centers and those who refer cases to the centers, and documentation clinically relevant biomarkers and translational research, are of paramount importance.

Overall, the approach we suggest could help in cutting costs and improving quality in both resource-poor and resourceful countries.

Materials and Methods

The study was initially performed on 296 cases in which a B-cell lymphoma or an HIV-associated lymphoma was suspected on initial morphological evaluation during the period from January 2012 to June 2012 at the Department of Histopathology of Hammersmith Hospital, Imperial College Healthcare NHS Trust, London, UK. Following this, the study was extended to and validated with an additional 516 cases of suspected B-cell lymphomas seen during the period between January 2005 and June 2012 at the Department of Medical Biotechnologies of the University of Siena, Italy. All 812 cases had been fixed in formalin and embedded in paraffin. Cases where the initial morphological description suggested Hodgkin lymphoma or a T-cell lymphoma were excluded.

Extensive immunohistochemical work-up had been undertaken on these cases. Where required, in-situ hybridization for Epstein-Barr virus-encoded RNA (EBER) and light chains, FISH analysis for MYC, BCL2, BCL6, and IG genes, and antigen receptor gene rearrangement studies had been undertaken. Initially, reports were evaluated, and each of the reports had concise, but meticulous morphological description and clear documentation of all the additional work-up undertaken.

The diagnostic process was employed by two phases, phase 1 and phase 2. Based on the morphological description (in cases other than those where HIV status was known), an attempt was made to slot cases into categories under phase 1. Cases that could not be placed into any of the categories under phase 1 were then assigned to diagnostic algorithms based on the morphological description. Biopsies from HIV-positive patients were placed under Algorithm 5.

Phase 1 Diagnosis

We identified six types of the B-cell lymphomas that had very characteristic morphology and thus required a short panel of immunostains to confirm the diagnosis (Table 1). These included the following:

1. Suspected chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL): characteristic cytological features and presence of proliferation centres. Confirm with CD20 (positive), CD5 (positive), CD23 (positive), CD10 (negative), and cyclin D1 (negative). If CD23 is negative, evaluate for SOX11 or proceed to Algorithm 2 in phase 2. Please note that if flow cytometry/immunophenotyping has documented the characteristic immunophenotype of CLL, lymph node biopsy and immunohistochemistry would not be essential. Suggested algorithms (both on phase 1 and 2 for CLL) are primarily for those cases where peripheral blood/bone marrow immunophenotyping has either not been performed or has not been informative.

2. Suspected follicular lymphoma, grades 1 to 3a (FL1-3a): a prominent follicular pattern and with presence of centrocytes accompanied by variable numbers of centroblasts. Confirm with CD20 (positive), CD10 (positive), BCL2 (positive), and cyclin D1 (negative). If features are not diagnostic, the case would need further evaluation by Algorithm 1 in phase 2.

3. Suspected follicular lymphoma, grade 3b (FL3b): a prominent follicular pattern and with infiltrate entirely composed of centroblasts (absence of centrocytes). Confirm with CD20 (positive), BCL6 (positive), BCL2 (positive), IRF4/MUM1 (positive) and cyclin D1 (negative). In follicular lymphoma with a follicular pattern (irrespective of grade), CD21 immunostain is recommended only when the...
nodular pattern is subtle or is only partially identified. In the latter case, quantifying diffuse and follicular components would be aided by CD21 stain. If CD21 demonstrates absence of follicular dendritic cell meshworks, such areas need to be evaluated for (diffuse large B-cell lymphoma) DLBCL.

4. Suspected mantle cell lymphoma (MCL): characteristic mantle zone pattern and monomorphic centrocyte cytology. Confirm with CD20 (positive), CD5 (positive), and cyclin D1 (positive). If cyclin D1 is negative, evaluate for SOX11 or proceed to Algorithm 2 in phase 2.

5. Suspected DLBCL: presence of sheets of large cells with morphology of centroblasts or immunoblasts. Confirm with CD20 (positive), CD5 (negative), CD21 (absence of follicular pattern) and Ki67 (high proliferation rate, >50%). CD5+ cases would need further evaluation for cyclin D1 expression. If CD21 highlights a follicular pattern, follicular areas would need evaluation by Algorithm 1 in phase 2.

6. Suspected Burkitt lymphoma (BL): diffuse sheets of monomorphic medium-sized cohesive cells with multiple paracentrally located nucleoli (score 3 morphology according to the algorithm proposed by Naresh et al1), starry sky pattern, and high mitotic and apoptotic rate. Confirm with CD20 (positive), CD10 (positive), BCL2 (negative), terminal deoxynucleotidyl transferase (negative), and cyclin D1 (negative). If morphology or immunophenotype was found unusual for BL, the case would proceed to Algorithm 4 in phase 2.

Phase 2 Diagnosis
These included B-cell lymphomas that did not have characteristic morphological features to be slotted into one of the phase 1 categories; immunohistochemistry results that did not conform to the characteristic patterns described in phase 1, though morphological features led to a short panel of immunostains; and morphology and clinical information that dictated they should go through different algorithms or decision trees.

Algorithm 1
Nodular pattern—cases with easily identifiable nodular pattern. The nodular pattern includes various follicular patterns and other “nodular” patterns mimicking a follicular pattern. Infiltrate could be monomorphic or polymorphic and cell type could be variable. The algorithm addresses the segregation of follicular lymphoma (FL), MCL, and marginal zone lymphoma (MZL). It also identifies rare CLL/SLLs with prominent proliferation centers mimicking a nodular pattern Figure 1.

Algorithm 2
Diffuse/interfollicular pattern with small to medium-sized cells. CD21 immunostain is used to exclude presence of nodular/follicular pattern. Cases with focal follicular pattern would be assessed by Algorithm 1. The number of large nucleolated cells should be <20% of the overall population of cells or <15 per high-power field. If the proportion of large cells is bigger, the case should be assessed for DLBCL. If the proportion of large cells is bigger, and CD21 identifies a nodular pattern, the case should be assessed both for DLBCL and by Algorithm 1. Algorithm 2 addresses the segregation of CLL/SLL, MCL, MZL, diffuse FL, and lymphoplasmacytic lymphoma (LPL) Figure 2.

Algorithm 3
Diffuse blastic infiltrates of medium-sized cells. Diffuse sheets of monomorphic medium-sized cells with high mitotic rate and/or fine chromatin. Algorithm 3 addresses the segregation of B-lymphoblastic lymphoma, blastoid MCL, and BL Figure 3. It also allows exclusion of T-lymphoblastic lymphomas and other mimics of lymphoblastic lymphoma.

Algorithm 4
Cases with features overlapping between BL and DLBCL. This is addressed using the previously published BL diagnostic algorithm Figure 4.

Algorithm 5
Biopsies from HIV-positive patients were directly evaluated by Algorithm 5. Algorithm 5 addresses the segregation of BL, DLBCL, BL/DLBCL, plasmablastic lymphoma, primary effusion lymphoma and human herpesvirus 8-associated large cell lymphoma in multicentric Castleman disease Figure 5.
All results available on the cases were entered into a database. Each case was then systematically analyzed for the adequacy of the confirmatory immunohistochemistry panel (phase 1) and the applicability of different diagnostic algorithms (phase 2). The minimal number of antibodies/tests that was essential to arrive at a specific diagnosis was

**Figure 1** Diagnostic algorithm for cases with a nodular pattern. Nodular pattern: follicular lymphoma (FL) vs mantle cell lymphoma (MCL) vs marginal zone lymphoma (MZL). If nodularity is subtle or seen only partially, the pattern must be confirmed/quantified by CD21 and/or CD23. Diffuse areas to be assessed for percentage of large cells and large cells per high-power field. If MZL with follicular colonization is suspected, perform: light chains, CD43, IgM, and IgD. Questions marks indicate the possibility of diagnosing cases of lymphoma with atypical immunophenotype. CLL, chronic lymphocytic leukemia.

**Figure 2** Diagnostic algorithm for cases with a diffuse an interfollicular pattern, and where the infiltrate is composed of small to medium-sized cells. Diffuse/interfollicular pattern with small to medium-sized cells: chronic lymphocytic leukemia (CLL) vs mantle cell lymphoma (MCL) vs mantle zone lymphoma/lymphoplasmacytic lymphoma. Diffuse pattern must be confirmed by absence of CD21/CD23+ follicular dendritic cell meshworks (if a nodular pattern is recognized, assess as for nodular pattern. Large cells should be <20% or <15/high-power field. CD20−, CD5+, and cyclin D1− cases: evaluation for T-cell lymphomas. In CD10+ and BCL6− cases, perform terminal deoxynucleotidyl transferase.
**Figure 3** Diagnostic algorithm for cases with a diffuse infiltrate of blastic cells. Diffuse blastic infiltrates of medium-sized cells: B-lymphoblastic lymphoma (BLL) vs T-lymphoblastic lymphoma (TLL) vs blastoid mantle cell lymphoma (MCL) vs Burkitt lymphoma. TdT, terminal deoxynucleotidyl transferase.

**Phase 1**
- Morphology (0-3)
  - BCL2 (–, 2; weak, 1)
  - CD10 (+, 1)
- Cumulative score:
  - ≥5-6, BL; 3-4, BL not excluded; <3, not BL

**Phase 2**
- Morphology (0-3)
  - BCL2 (–, 2; weak, 1)
  - CD10 (+, 1)
  - Ki-67 (≥95%, 2; 90%-95%, 1)
  - CD38 (+, 1)
  - CD44 (–, 1)
- Cumulative score:
  - ≥8, BL; 6-7, BL not excluded; <6, not BL

**Phase 3**
- Morphology (0-3)
  - BCL2 (–, 2; weak, 1)
  - CD10 (+, 1)
  - Ki-67 (≥95%, 2; 90%-95%, 1)
  - CD38 (+, 1)
  - CD44 (–, 1)
  - FISH (MYC-lg+ and BCL2/BCL6–, 2)
- Cumulative score:
  - ≥8, BL; 6-7, BL not excluded

**Figure 4** Diagnostic algorithm for cases where morphological features overlap between Burkitt lymphoma (BL) and diffuse large B-cell lymphoma. CD20+ and terminal deoxynucleotidyl transferase–negative tumor with a diffuse infiltrate of medium to large lymphoid cells. Benefit from karyotype, comparative genomic hybridization, gene expression, and assessment of the impact of each of the parameters. FISH, fluorescence in situ hybridization.

**Phase 1**
- Morphology (0-3)
  - CD20 (–, 2; weak, 1)
  - CD10 (+, 1)
- Cumulative score:
  - ≥5-6, BL; 3-4, BL not excluded; <3, not BL

**Phase 2**
- Morphology (0-3)
  - CD20 (–, 2; weak, 1)
  - CD10 (+, 1)
  - CD38 (+, 1)
  - CD44 (–, 1)
  - PAX5 (–, 2; 60%-65%, 1)
- Cumulative score:
  - ≥8, BL; 6-7, BL not excluded; <6, not BL

**Phase 3**
- Morphology (0-3)
  - CD20 (–, 2; weak, 1)
  - CD10 (+, 1)
  - CD38 (+, 1)
  - CD44 (–, 1)
  - FISH (MYC-lg+ and BCL2/BCL6–, 2)
- Cumulative score:
  - ≥8, BL; 6-7, BL not excluded

**Figure 5** Diagnostic algorithm for lymphoma cases in the HIV setting. Aggressive B-cell lymphoma in HIV setting: Burkitt lymphoma (BL), BL/diffuse large B-cell lymphoma (DLBCL), plasmablastic lymphoma (PBL), primary effusion lymphoma (PEL), and human herpesvirus 8–associated large B-cell lymphoma (LBCL) in multicentric Castleman disease (MCD). EBER, Epstein-Barr virus–encoded small RNA.
Results

Among the 812 cases, 446 (55%) were evaluated initially by phase 1; 366 cases could not be placed under any of the categories of phase 1 and went through phase 2 algorithms.

Phase 1: Overall, in 89% of cases (398/446 cases), correct diagnosis was achievable based on the suggested panels. This included 83% of 241 suspected DLBCLs, 95% of 129 suspected cases of FL1-3a, 100% of 56 suspected CLL and others as described. Thirty-two DLBCLs expressed CD5, which necessitated cyclin D1 immunohistochemistry. Five cases of FL1-3a were CD10 negative, and three cases of FL1-3a were BCL2 negative. These cases also required further immunostains to arrive at the correct diagnosis. The initially suspected diagnosis on morphology was incorrect in nine (4%) of 446 cases, and these included seven T-cell lymphomas and two blastoid MCLs Table 2.

Phase 2: Overall, of 366 cases analyzed in phase 2, 41% required five or fewer, and 93% required eight or fewer antibodies to arrive at a specific diagnosis. Among the 159 cases with a nodular pattern, 38% required five or fewer, and 92% required eight or fewer antibodies to arrive at a specific diagnosis. Among the 120 cases with a diffuse interfollicular pattern, 52% required five or fewer, and 100% required eight or fewer antibodies to arrive at a specific diagnosis. Among the 14 HIV+ lymphomas, 57% required five or fewer, and 93% required eight or fewer antibodies/tests to arrive at a specific diagnosis Table 3. Proportion of each of the different lymphomas among cases with different morphologic patterns is provided in Table 4.

Overall, 799 B-cell lymphomas were evaluated. DLBCL and FL1-3a were the most frequent lymphomas Table 5. A correct diagnosis was achievable in 69% cases with the application of three to five antibodies. Furthermore, a correct diagnosis was achievable in 90% cases and 96% cases using seven antibodies and eight antibodies, respectively Table 6.

Discussion

Through this study, we document that in the hands of well trained hematopathologists, dependable diagnosis of B-cell lymphomas is achievable, even under considerable economic constraints. It should be stressed that this cannot be achieved without thorough knowledge and experience in morphology of lymphoid lesions, and without a good understanding of the current WHO classification of lymphoid neoplasms. Without a good knowledge base and expertise, the choice of initial panels and the subsequent decisions made via the algorithms can be compromised. While using limited panels, there would be little levy for suboptimal technical abilities in tissue fixation, processing or immunohistochemistry. Hence, these limited panels are likely to be useful only in the setting of a large-volume lymphoma practice that would ensure good technical quality and contribute to substantial expertise among the reporting hematopathologists. The quality of H&E-stained slides would also be crucial.

Using an algorithmic approach poses certain issues:

1. It would be difficult to use this approach in cases requiring a stringent TAT. The very nature of dichotomized decision making before progressing to the next panel extends the TAT.
2. By using short panels, one loses the safety net of using multiple overlapping antibodies to arrive at similar conclusions. For example, if one used CD20 in isolation instead of CD20, CD79a and PAX5, it is possible that a B-cell lymphoma with weak/absent CD20 expression might be missed (or sample from a patient on rituximab therapy, when information is not provided).
3. It hampers continued growth of hematopathologists.

Due to these issues, the proposed algorithmic approach needs to be introduced with caution, and only where necessary, ie dictated by extraordinary economic constraints.

The rationale for incorporating specific antibodies within the algorithm in specific situations is summarized as follows:

1. In our studies on material from developing countries, we have found preservation of nuclear antigens such as BCL6 to be suboptimal. Hence, CD10 has been preferred over BCL6 in most phase 1 algorithms (with exception of FL3b, where CD10 is frequently not expressed). Furthermore, BCL6 is expressed on T-cells within follicles.
2. CD20 is required to establish the B-cell nature of the disease and also to provide confidence to the clinicians to use rituximab if deemed necessary.
3. BCL2 would be essential in FLs to exclude hyperplastic follicles mimicking FL.
4. CD21 has been used for identification of follicular architecture and for quantification of follicular and diffuse components.
5. With respect to CLL, appreciation of proliferation centers depends on the quality of H&E sections. Prominence of proliferation centers is variable between cases. Even in cases with identifiable proliferation centers, BCL2 would be essential in FLs to exclude hyperplastic follicles mimicking FL.
centers, they can be easily missed in poor-quality preparations, which are not uncommon in resource-poor countries. Hence, a panel that would take help arriving at a specific diagnosis has been designed.

6. Without the use of Ki67, there is a chance of overdiagnosis of DLBCL in suboptimal-quality material in cases of FL with diffuse areas and MZL with relatively prominent large cells. Here Ki67 acts as a safety net.

7. In FL3b, BCL2 could be negative. In such cases, strong expression of MUM1 is often helpful. Furthermore, MUM1 expression dichotomizes FLs into FL grades 1 to 2 and FL grade 3. Cyclin D1 is used in the panel to differentiate from blastoid and pleomorphic variants of MCL with a nodular pattern.

Despite the above reservations, this approach will help in usage of the WHO classification of lymphoma in large parts of the world with limited resources, as well as impact entry into and analysis of patients in locally relevant clinical trials will be feasible. Without classifying lymphomas correctly, treatment outcomes cannot be measured meaningfully.

Recently, antibodies such as LEF1 have been introduced and appear to be very specific for CLL.9 Similarly, utility of antibodies such as IRTA1 and MNDA are being investigated in the diagnosis of MZL.10 Diagnostic algorithms may need further modifications with introduction of these “new” antibodies to clinical practice. As a spin-off from this study, we can also propose a limited panel of antibodies to be used in units referring suspected lymphoma cases (units that do not make the final diagnosis) to specialist referral centers. This pertains to networks such as those in the United Kingdom, where lymphoma diagnosis is centralized to SIHMDS. In many such networks, a general pathologist initially evaluates the lesion, performs immunohistochemistry and forwards cases of lymphoma or those suspicious of lymphoma to the

| Table 2 |

Diagnostic Ability at Phase 1

<table>
<thead>
<tr>
<th>Suspected Category on Morphology (No.)</th>
<th>% (No. of) Cases That Reached Correct Diagnosis</th>
<th>% (No. of) Cases Requiring Additional Work-up to Reach Correct Diagnosis</th>
<th>% (No. of) Cases Where Correct Diagnosis Was Not Suspected on Morphology</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLL (56)</td>
<td>100% (56)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>FL1-3a (129)</td>
<td>95% (122)</td>
<td>5 (7)</td>
<td>3 were CD10 negative</td>
</tr>
<tr>
<td>FL3b (4)</td>
<td>100% (4)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MCL (9)</td>
<td>100% (9)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DLBCL (241)</td>
<td>83% (200)</td>
<td>13% (32)</td>
<td>4% (9)</td>
</tr>
<tr>
<td>BL (7)</td>
<td>100% (7)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total (446)</td>
<td>89% (398)</td>
<td>0</td>
<td>2% (9)</td>
</tr>
</tbody>
</table>

BL, Burkitt lymphoma; CLL, chronic lymphocytic leukemia; DLBCL, diffuse large B-cell lymphoma; FL1-3a, follicular lymphoma grades 1-3a; FL3b, follicular lymphoma grade 3b; MCL, mantle cell lymphoma.

| Table 3 |

Minimum Number of Antibodies That Would Have Been Required to Arrive at the Correct Diagnosis in Phase 2

<table>
<thead>
<tr>
<th>Pattern/Algorithm (No.)</th>
<th>3 Antibodies</th>
<th>4–5 Antibodies</th>
<th>6–8 Antibodies</th>
<th>9–10 Antibodies/Tests*</th>
<th>&gt;10 Antibodies/Tests*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nodular (159)</td>
<td>8% (13)</td>
<td>30% (47)</td>
<td>54% (86)</td>
<td>4% (6)</td>
<td>4% (7)</td>
</tr>
<tr>
<td>Diffuse/interfollicular pattern (120)</td>
<td>12% (14)</td>
<td>40% (48)</td>
<td>48% (58)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Diffuse blastic pattern (26)</td>
<td>77% (20)</td>
<td>23% (6)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>BL/DLBCL algorithm (47)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>HIV lymphomas (14)</td>
<td>7% (1)</td>
<td>50% (7)</td>
<td>36% (5)</td>
<td>—</td>
<td>7% (1)</td>
</tr>
<tr>
<td>Total (286)</td>
<td>8% (28)</td>
<td>33% (122)</td>
<td>52% (189)</td>
<td>4% (15)</td>
<td>3% (12)</td>
</tr>
</tbody>
</table>

BL, Burkitt lymphoma; CLL, chronic lymphocytic leukemia; DLBCL, diffuse large B-cell lymphoma

*Some tests included fluorescent in situ hybridization analysis for MYC, BCL2, BCL6 and IG gene rearrangements. In some cases, it included in situ hybridization for Epstein-Barr virus (EBV)-encoded small RNA (EBER) (EBV detection).
SIHMDS. At the SIHMDS, original slides are reviewed, and additional immunostains (with some repeat of immunostains) are undertaken with or without investigations pertaining to FISH and molecular diagnostics before a final diagnosis is issued. This two-stage reporting can lead to (a) duplication of work in terms of immunohistochemistry; (b) lack of clarity in the extent to which the case should be investigated at the referring center; (c) delay in referral; and (d) depletion of tissue in the paraffin block in small biopsies before the referral.

Some of the essential antibodies such as PAX5 (in the diagnosis of classical Hodgkin lymphoma) have not been included in the algorithms. Such essential antibodies would be used at the referral center before treatment is initiated.

Our proposal for referral of lymphoid lesions in the context of SIHMDS networks includes the following:

1. Not to perform immunostains in small or needle core biopsies; paraffin block should be referred to SIHMDS on evaluation of the initial H&E section, and paraffin block should not be subjected to any additional levels.
2. Though a limited panel of immunostains may be performed, referral should be made within 72 hours of the receipt of biopsy.
3. Suggested short panels on adequate biopsies include the following (especially if one is working under economic constraints):
a. Suspected “small” cell lymphoma or lymphoma with a follicular pattern: CD20, CD5, CD10, BCL2, CD23, and cyclin D1

b. Suspected lymphoma with medium- to large-sized cells and with a diffuse pattern: CD20, CD3, CD43, and Ki67


The above panels should be adequate to separate reactive lymphoid lesions and nonhematopoietic lesions, and to enable targeted referrals to SIHMDS. This would also allow maintaining a certain level of lymphoma diagnostic skills in the referring units.

To conclude, the study and the panels suggested intend to (1) allow well trained hematopathologists to practice lymphoma diagnostics in countries with limited resources; and (2) referral pathways to function in the context of SIHMDS networks in countries such as the UK. These algorithms are not intended to be part of a standard operating procedure in specialized diagnostic services such as SIHMDS.

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References


