A web-based database for diagnosis of haematologic neoplasms using immunophenotyping by flow cytometry

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Abstract. The interpretation of immunophenotyping results by flow cytometry involves pattern recognition of different haematologic neoplasms that may have similar immunologic marker patterns. The numerous markers available in the flow cytometry laboratory make these patterns difficult to remember, especially for those of uncommon neoplasms. We describe the design and implementation of a Web-based database for diagnosis of haematologic neoplasms using results of immunophenotyping by flow cytometry. This database aims to assist pathology and haematology residents in interpreting flow cytometry data, and is designed to reach a wide base of users who use a variety of browsers on different computer platforms. Five modules are developed in this comprehensive program: (a) differential diagnosis: to generate a list of differential diagnoses that closely match the marker results in a given case; (b) display of disorders: typical results of markers for each disorder; (c) display of markers: relevant information of each immunologic marker; (d) display of archived cases for a disorder: marker results of cases previously diagnosed for a disorder; and (e) display of summary for archived cases: summary of marker results of all the archived cases for each disorder. Our experience with this Web-based database in teaching pathology residents has been very encouraging. Since the World Wide Web is increasingly more accessible to computer users, it has become an ideal medium for distribution of clinical decision-support software.

Keywords: World Wide Web; Haematologic neoplasms; Relational database; Immunophenotyping; Flow cytometry.

1. Objectives

Immunophenotyping has become one of the essential methods for proper classification of haematologic neoplasms. Flow cytometry used in immunophenotyping has undoubtedly added a new dimension to the diagnosis of leukaemia and lymphoma [1,2]. A wide range of monoclonal antibodies is currently available to recognize various haematologic cells based on their surface and cytoplasmic antigens [1–4]. Leukaemic and lymphoma cells cannot usually be detected with a single immunologic marker. Instead, the use of a monoclonal antibody panel consisting of multiple antibodies is required to support the provisional diagnosis based on histological findings [1,2]. Since many haematologic neoplasms demonstrate similar patterns of immunophenotyping

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[2–4], their diagnosis often presents a challenge to pathologists and haematologists who interpret flow cytometric data. This is particularly applicable to pathology and haematology residents in training as well as practicing pathologists who are not subspecialized in haematopathology. As the number of immunologic markers used in flow cytometry increases, a systematic approach in interpretation of marker results is also essential for consistent classification of neoplasms [5].

The interpretation of immunophenotyping results by flow cytometry involves pattern recognition of different haematologic neoplasms that may have similar immunologic marker patterns. Each neoplasm is associated with a particular pattern characterized by the presence of certain markers and absence of others [2–4]. The numerous markers available in the flow cytometry laboratory make these patterns difficult to remember, especially for those of uncommon neoplasms [2–4]. Another factor that also hinders the interpretation process is the lack of consistency in marker results for a particular neoplasm [2–4]. A certain marker may be positive (or negative) for a certain neoplasm in most of the cases. However, exceptions are often seen. For this reason, an absolute diagnostic pattern is usually not available for each specific neoplasm. Instead, the diagnostic approach is to seek a neoplasm with marker pattern that closely matches the given marker results [2]. Since the immunophenotyping pattern of haematologic neoplasms can easily be described in terms of the presence or absence of markers listed in separate fields, a database is a logical approach to facilitate the interpretation of marker results. In addition, a decision-support system that readily displays the attributes associated with a specific neoplasm would be very convenient and useful to pathologists and haematologists who encounter some uncommon diseases from time to time.

A relational database for interpretation of immunophenotyping by flow cytometry, CD-Marker, had previously been developed by the authors to teach pathology residents at our institution [6]. CD-Marker was implemented in Microsoft Access 97 (Microsoft Corp., Redmond, Washington, USA) and was run on an IBM-compatible computer under Microsoft Windows. To use CD-Marker, users had to install the run-time version of this database on their computer. The other option was to obtain a copy of this database file and open it with Microsoft Access 97. These requirements limited the number of users of this stand-alone database.

The World Wide Web (WWW) offers a simple solution to this problem by providing easy access to online materials. Existing internet networks across multiple platforms can be used as the medium for software implementation. Users located in any part of the world with an internet connection can use browsers to get access to online materials that reside in centralized web servers. A number of websites have been dedicated to different topics in immunophenotyping using flow cytometry [7–11]. Those are usually developed by academic institutions for teaching [7–9] and by commercial vendors [10,11] for advertising their products. While a great deal of valuable information can be retrieved from these sites on many topics, their contents are not comprehensive enough to be used as teaching materials for our pathology residents who interpret flow cytometry results.

In this article, we describe the design and implementation of a web-based database for interpretation of immunophenotyping by flow cytometry at our institution and discuss the potential role of this program in medical education as

well as in clinical consultation. This web-based program is designed to reach a wide base of users who use a variety of browsers on different computer platforms. The interface of this program should be intuitive to an average user who is not required to be computer-savvy. The hardware requirement to use the program should be at a minimum to allow adequate performance on most users' computer.

2.   Methods
CD-MarkerDX, a web-based database for interpretation of immuno-phenotyping by flow cytometry, is implemented in three tiers: presentation layer, logics layer, and data layer.

1. Presentation layer: the graphic interface with which the user interacts. It presents data and captures the user's input.
2. Logics layer: a set of software components that perform analysis on data.
3. Data layer: a relational database containing the domain data.

A major challenge in designing dynamic databases on the web has been to accommodate various types of web browsers that use different client-side technology (Client-side Active X, different Java versions, etc.). Microsoft Active Server Pages (ASP) technology helps facilitate development of browser-independent databases on the WWW [12,13]. All the processing work is done on the web server, allowing for the use of a 'thin' client (a web browser without any plug-ins or extensions).

The ASP technology works on the Active Server platform [12] that utilizes Component Object Model (COM). Manipulation of data can be achieved with Active Data Object (ADO) in conjunction with Structured Query Language (SQL). Some useful Active Server components are available from the COM library. Most Active Server components for the inference engine of CD-MarkerDX are built in this project with Microsoft Visual Basic, in the form of ActiveX dynamic link library (DLL) files, due to specific requirements. Stored SQL procedures in the database server ease the design of complex queries performed on the database. VBScript and JavaScript are used for scripting on the server side and the client side [14,15], respectively. CD-MarkerDX is installed on a Microsoft Windows NT 4.0 server running Microsoft Internet Information Server 4.0. The data reside in a Microsoft SQL Server 7.0 database. Active Server components are managed by Microsoft Transaction Server 2.0 for scalability.

The core materials in CD-MarkerDX are derived from those developed for the differential diagnosis module of CD-Marker, a Microsoft Access 97 database for personal computers previously designed by the authors [6]. A total of 33 haematologic neoplasms, and 43 immunologic markers (table 1) are included in the knowledge base of CD-Marker. A complete listing of immunophenotype for each neoplasm is included in the appendix. The marker result is designated as positive (or negative) for a neoplasm if more than 80% of the cases are found to be positive (or negative) for that marker. The search engine in this program, written in Microsoft Visual Basic for Application, is based on backward-chaining inference [16–18]. CD-Marker has been tested with 92 clinical cases from two

Table 1. List of immunologic markers in the knowledge base.

CD1	CD38
CD2	CD41
CD3	CD42
CD4	CD43
CD5	CD45
CD7	CD56
CD8	CD57
CD10	CD61
CD11b	CD71
CD11c	CD77
CD13	CD79a
CD14	CD79b
CD15	CD103
CD16	HLA-DR
CD19	Monoclonal sIg (surface light chain restriction)
CD20	Monoclonal cIg (cytoplasmic light chain restriction)
CD21	PC-1
CD22	TdT
CD23	Cytokeratin
CD24	Glycophorin A
CD25	Co-expression of CD5 and CD19
CD33	FMC-7

tertiary medical centres. These are patient cases at Lyndon B. Johnson Hospital and Ben Taub Hospital (Harris County Hospital District, Houston, Texas, USA). Flow cytometry study for these cases was performed between January 1995 and December 1996. Immunophenotyping by flow cytometry was performed on a FACScan (Becton Dickinson, Mountain View, CA, USA). The specimens in our cases included bone marrow, lymph node, spleen, body fluid, and extranodal haematologic tumours. Data for these 92 cases were retrospectively retrieved and immunophenotyping data were tested on CD-Marker. The number of cases for each disorder is shown in table 2. The final diagnosis of each case was previously established by histological findings and correlation with flow cytometry results. The final diagnosis was documented in surgical pathology reports including bone marrow reports. Data entry of each case for CD-Marker included only marker results that were available in the flow cytometry laboratory at Ben Taub Hospital at the time of initial presentation. Only definitive marker results (positive or negative) in each case were used in validation. Equivocal results were not used due to their lack of contribution to the validation results. To avoid potential bias in the design of CD-Marker, its knowledge base has been developed by one of the authors (A.N.) who had not interpreted the cases previously. CD-Marker ranked the actual diagnosis as one of the top five differential diagnoses in 93% of the cases tested (table 3). Further details of the components, inference algorithm, and validation results for CD-Marker have previously been described [6]. Since the same inference algorithm is used in CD-Marker and CD-MarkerDX, the validation results for CD-Marker are also applicable to CD-MarkerDX. Conversion of the software from the Access-based version to the web-based version requires complete rewriting of all the software codes to accommodate the web medium. Figure 1 illustrates the relationship between the three organizational layers, showing how CD-MarkerDX is built using web browser at the presentation layer, Internet

Table 2.   Number of cases used in validation (total 92 cases).

Disorder	Number of cases
Follicular small cleaved B-cell lymphoma	5
Mantle B-cell lymphoma	2
Large B-cell lymphoma	5
Mediastinal B-cell lymphoma	2
B-cell lymphoma, unclassifiable, mixed small and large cells	2
Lymphoplasmacytoid B-cell lymphoma	2
Marginal B-cell lymphoma	3
Burkitt's B-cell lymphoma/ Acute lymphoblastic B-cell leukaemia, L3	3
Splenic B-cell lymphoma with villous lymphocytes	4
Peripheral T cell lymphoma	5
Lymphoblastic lymphoma (T cell)	2
Thymoma	2
Chronic lymphocytic leukaemia (B cell)/Small lymphocytic lymphoma	7
Chronic lymphocytic leukaemia (T cell)	2
Prolymphocytic leukaemia (B cell)	2
Hairy B-cell leukaemia	4
Sezary syndrome/ Mycosis fungoides	2
Adult T cell leukaemia/ lymphoma	2
Acute lymphoblastic leukaemia (T cell precursor)	2
Acute myeloblastic leukaemia without maturation, M1	3
Acute myeloblastic leukaemia with maturation, M2	4
Acute promyelocytic leukaemia, M3	2
Acute myelomonocytic leukaemia, M4	2
Acute monoblastic leukaemia, M5	2
Acute erythroleukaemia, M6	2
Acute megakaryoblastic leukaemia, M7	2
Biphenotypic acute leukaemia, AML & T cell ALL	2
Biphenotypic acute leukaemia, AML & B cell precursor ALL	2
Large granular lymphocyte leukaemia, NK cell	2
Large granular lymphoproliferative disorder, T cell	2
Acute lymphoblastic leukaemia (Early-B precursor)	2
Acute lymphoblastic leukaemia (CALLA)	3
Acute lymphoblastic leukaemia (Pre-B)	4

Table 3.   Summary of the validation results.

Ranking	Number of cases	Percentage	Accumulated percentage
First differential diagnosis	39	42	42
Second differential diagnosis	23	25	67
Third differential diagnosis	12	13	80
Fourth differential diagnosis	10	11	91
Fifth differential diagnosis	2	2	93
Lower ranking	6	7	–
Total	92	100	–

Information Server and Transaction Server at the logics layer, and Database Server at the data layer. The database's main menu has five modules:

1. Differential diagnosis: to generate a list of differential diagnoses that closely match the marker results in a given case;
2. Display of disorders: typical results of markers for each disorder;
3. Display of markers: relevant information on each immunologic marker;
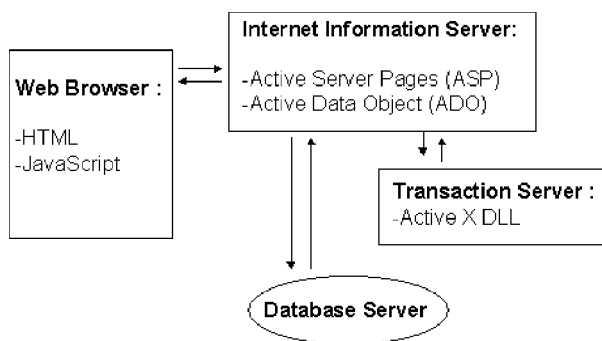
Figure 1.   Components of the web-based database.

4. Display of archived cases for a disorder: marker results of cases previously diagnosed for a disorder;
5. Display of summary for archived cases: summary of marker results of all the archived cases for each disorder.

The graphic user interface for the differential diagnosis module is shown in figure 2. All the available marker results should be entered for the case under consideration. Lack of information in certain data fields does not prevent CD-MarkerDX from processing the data. However, the accuracy of the suggested diagnosis will be compromised if results of important markers are left out. A list of differential diagnoses is provided by CD-MarkerDX with each set of input data. The differential diagnoses have an assigned value of certainty factor (C). The C value for a neoplasm reflects how well its immunophenotyping pattern matches the marker data in a given case. This factor is defined as:

$$C = \frac{M}{M + N} \tag{1}$$

where C=certainty factor for a particular neoplasm $(0 \leqslant C \leqslant 1)$; M=the number of attributes of a neoplasm that match the input data; and N=the number of attributes of a neoplasm that do not match the input data.

Note that the value of C, as defined in the equation above, only reflects the similarity between a neoplasm's attributes and the available data. A high value of C for a neoplasm, such as 1, does not exclude the possibility that more data input with increased value of N may eventually decrease its C value.

3. Results

To demonstrate how the knowledge base of CD-MarkerDX is implemented, the diagnostic criteria for chronic lymphocytic leukaemia/small lymphocytic lymphoma (B-cell CLL/SLL) are given below as an example [2–4]:

1. The malignant cells are positive for the following markers: CD5, CD19, CD20, CD21, CD23, CD24, CD43, CD79a, HLA-DR, sIg, and coexpression of CD5 and CD19 or CD20.
2. The malignant cells are negative for the following markers: CD2, CD3, CD4, CD7, CD8, CD10, CD25, and FMC7.

Figure 2.   Input data for a case of B-cell chronic lymphocytic leukaemia.

A summary of diagnostic attributes for B-cell CLL/SLL is shown in figure 3. Note that some marker results are left blank in this set of attributes. Only the markers considered to play an important role in differential diagnosis are included. This design helps to minimize the computing time in the inference process.

When marker results of a given case are entered, they are processed by the database inference engine and a list of differential diagnoses will be displayed. These diagnoses are listed with their associated C value. A demonstration of a case with B-cell CLL/SLL illustrates how CD-MarkerDX can be used to interpret immunophenotyping results and how its search mechanism works. Figure 2 shows the marker data available for the patient sample. CD-MarkerDX attempts to match this set of data with the diagnostic attributes of 33 haematologic neoplasms in the knowledge base. The available data match the following attributes of B-cell CLL/SLL:

1. Positive for: CD5, CD19, CD20, CD23, HLA-DR, sIg, and coexpression of CD5 and CD19.
2. Negative for: CD3, CD4, CD7, CD8, CD10, and CD25.

The total number of attributes of B-cell CLL/SLL that match the input data is 13 (seven positive results and six negative results). This number is represented by the variable M in Equation 1 (M=13). None of the attributes of B-cell CLL/SLL is in conflict with the input data (N=0). Note that the following input data do not have a corresponding attribute for B-cell CLL/SLL in the knowledge base:
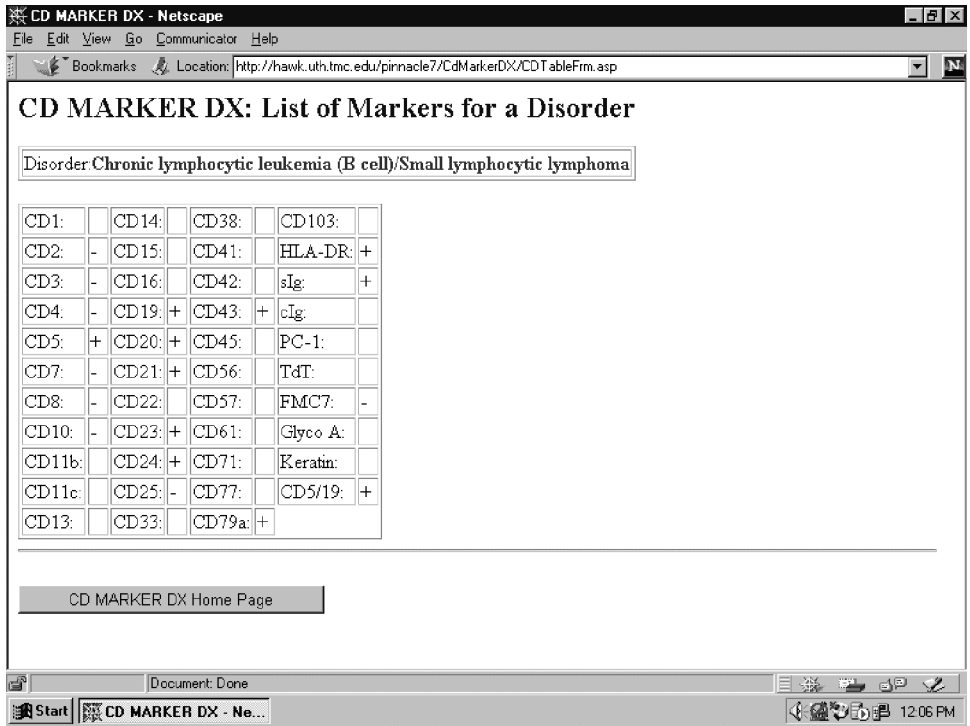
Figure 3.   Immunophenotyping for B-cell chronic lymphocytic leukaemia/ small lymphocytic lymphoma.

CD11c, CD14, CD16, CD22, CD45, Glycophorin A, and Cytokeratin. These input data do not have any impact on the ranking of B-cell CLL/SLL since they are not included as part of the calculation of its C value. Similarly, the following attributes in the knowledge base without corresponding input data have no impact on the C value for B-cell CLL/SLL: CD2, CD21, CD24, CD43, CD79a, and FMC7. The intentional exclusion of input data without corresponding attributes in the database (or attributes without corresponding input data) in calculating the C value serves an important purpose of maintaining a flexible design for the knowledge-base as well as for the data input panel. Since different flow cytometry laboratories may utilize different markers in immunophenotyping and various studies on marker pattern of neoplasms have used different marker panels, an absolute requirement of certain markers in the interpretation process would be too stringent to yield any reasonable matches [5]. The C value for B-cell CLL/SLL at this point is:

$$C = \frac{13}{(13 + 0)} = 1 \tag{2}$$

After CD-MarkerDX calculates the C value for all the remaining 32 haematologic neoplasms in the knowledge base and ranks them accordingly, it lists the following leading diagnoses:

1. B-cell CLL/SLL; C=1
2. Prolymphocytic B-cell leukaemia; C=1

3. Mantle B-cell lymphoma; C=0.88
4. Mixed B-cell lymphoma; C=0.87
5. Large B-cell lymphoma; C=0.85

B-cell CLL/SLL and prolymphocytic B-cell leukaemia had the same C value (C=1) with the given input data. A second criterion is needed to refine the ranking process for neoplasms with the same C value. This second criterion is the difference between the matched attributes and the unmatched attributes for a neoplasm (M−N). With this additional criterion, B-cell CLL/SLL is ranked as the leading diagnosis (C=1, M−N=13), followed by prolymphocytic B-cell leukaemia (C=1, M−N=12). Figure 4 shows the list of differential diagnoses with all the calculation results. The search mechanism of going from neoplasms in the database to the input data for the best matches represents a strategy known as backward-chaining search [16–18].

This demonstration shows the open-ended format of the data input. The data panel consists of many immunologic markers, some of which may not be part of routine testing in certain laboratories. Consequently, the actual data input for a case are unlikely to account for all the markers in the data panel. However, the availability of essential data would influence the accuracy of ranking by CD-MarkerDX. Coming back to the demonstration case of B-cell CLL/SLL, the ranking results would be different if the result for CD5 and CD23 had not been available. In this scenario, prolymphocytic B-cell leukaemia would have been the leading diagnosis (C=1, M−N=12), followed by B-cell CLL/SLL (C=1, M−N=11).
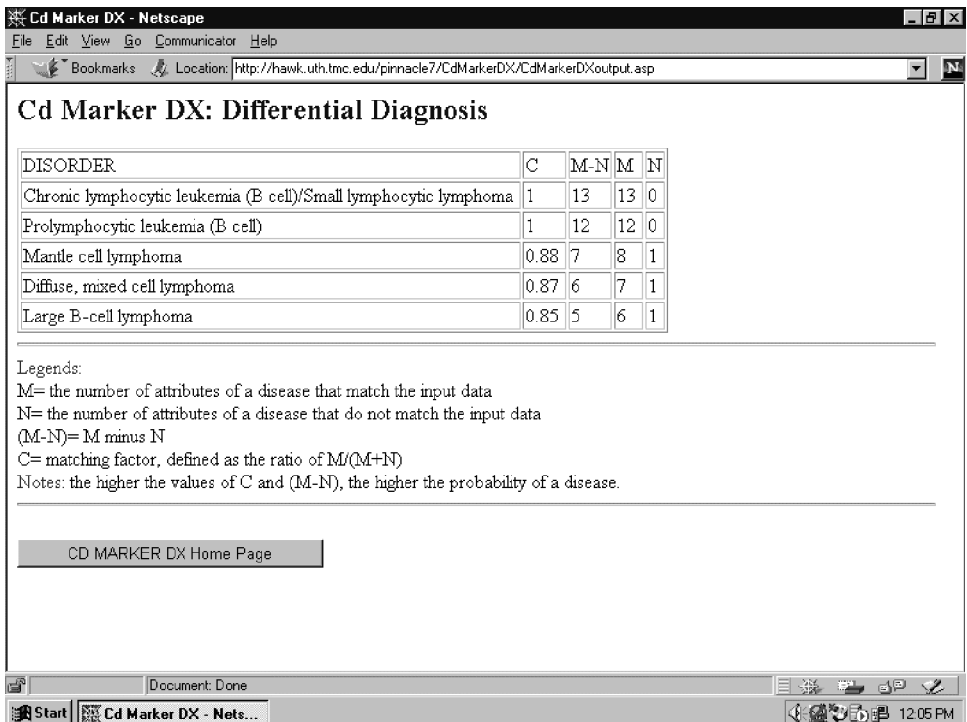

Figure 4. Output for the demonstration case of B-cell chronic lymphocytic leukaemia.

Laboratory data input may be subject to errors due to technical problems in laboratory testing and also subjective interpretation of results (a 'normal' value versus a 'borderline abnormal' value). For this reason, it is critical to take into account certain elements of uncertainty in data input while evaluating a clinical case. CD-MarkerDX offers the user the option of 'What If' reruns to enhance the flexibility in handling data that are not clear-cut. The reruns can be performed after the user edits one or more of the input data. This editing may be in the form of changing the value of the data (normal to abnormal or vice versa), or adding additional data. Without requiring different sets of data entry, the 'What If' reruns offer the user a convenient way to consider all the potential diagnoses based on laboratory data that may involve borderline values, subjective interpretations, or human errors.

The critical role of the interpreting pathologist or haematologist cannot be overemphasized. CD-MarkerDX is only useful in suggesting a list of differential diagnoses. The pathologist or haematologist must establish the final diagnosis by correlating the histological and clinical findings of the case with the immunophenotyping results. The immunologic marker pattern of neoplasms in the list of differential diagnoses can be reviewed during the interpretation process by using the 'Display of disorders' feature of CD-MarkerDX (figure 3). To compare the marker pattern of the current case with those of previously diagnosed cases (a total of 92 archived cases), the interpreting pathologist or haematologist can use the archival modules of CD-MarkerDX. The 'Display of archived cases for a disorder' module lists marker results of all the archived cases with a certain diagnosis. The 'Display summary for archived cases' module shows the number of archived cases (with a certain diagnosis) with a positive (or negative) result for each marker.

4. Discussion

We developed a web-based database for laboratory diagnosis of haematologic neoplasms using flow cytometry results. This program has been used as supplemental teaching material for pathology residents on clinical pathology rotations at our institution since 1998. This web-based program has been received with enthusiasm by our residents.

Bandwidth limitation has not been a problem for CD-MarkerDX in our experience. The current design of CD-MarkerDX allows for a minimum requirement of hardware and software. A low-end system (486 CPU, 16 megabytes of RAM, and a 14.4 K modem) has been found to be adequate for using CD-MarkerDX. This program has been tested with web browsers on various operating systems without any technical problems. As shown in the preceding demonstration, CD-MarkerDX is designed with a user-friendly interface. This graphic user interface is arranged such that the sequence of data entry, display of results, and review of marker pattern should be intuitive to the user.

This database provides a convenient, interactive tool to assist clinical personnel in diagnosing haematologic neoplasms using flow cytometric data. This web-based database is available to a large number of users via the WWW. As the web provides medical students and residents with convenient tools for self-study and clinical decision-support, web-based educational programs may eventually form the core material for life-long learning by physicians, especially at the point-of-service

[19–25]. Further studies would be needed to determine the most effective ways to utilize this new emerging medium in medical education.

Despite the utility of CD-MarkerDX, there are certain constraints inherent in its use. Some of these have previously been described for its predecessor, CD-Marker [6]:

(a) The user must have a functional knowledge of haematologic disorders to be able to use CD-MarkerDX effectively because this program only serves as a search tool to aid the user in making a diagnosis. The technical skills to perform the laboratory procedures and the experience needed to accurately gate the cellular populations are critical in the diagnostic process. CD-MarkerDX can generate a list of differential diagnoses in most cases if adequate data are input. However, it cannot be overemphasized that human judgment is the most important element in finalizing the diagnosis. The number and complexity of haematologic neoplasms require that the differential diagnoses suggested by CD-MarkerDX be reviewed before making a final diagnosis. We believe that the pathologist's or haematologist's clinical judgment and the information he or she gathers from CD-MarkerDX should yield an accurate diagnosis.

(b) The current version of CD-MarkerDX is deficient in handling some cases of T-cell malignancy due to the difficulty in designing an algorithm for detection of the random loss of T-cell antigens as previously discussed [2–4]. We are currently working on several approaches to alleviate this shortcoming and expect to implement a new technique to handle T-cell malignancies more effectively in future versions of CD-MarkerDX.

(c) CD-MarkerDX would not be useful in the diagnosis of neoplasms that traditionally have not been shown to benefit from flow cytometric immunophenotyping [2–4]. These include Hodgkin's disease, multiple myeloma, and cases of atypical immunophenotypes.

(d) The current version of CD-MarkerDX does not take into account the intensity of fluorescence signal detected in flow cytometry analysis (for example, light chain restriction is faint in B-cell CLL/SLL). The addition of this criterion for many markers (where applicable) is planned for the next version of this database and is expected to yield more accurate diagnosis.

(e) For certain neoplasms, a definitive diagnosis cannot be made without additional confirmatory tests after flow cytometry analysis. For example, diagnosis of mantle B-cell lymphoma should be confirmed by a cytogenetic result of translocation t(11; 14)(q13; q32) or detection of Cyclin D1 in the malignant cells. These additional tests are currently outside the scope of our database, which focuses solely on immunophenotyping.

(f) The current version of CD-MarkerDX does not allow the user to change contents of the database due to security reason. We plan to add this option to the program in the future to allow the user to customize the database contents to fit individual needs.

5. Conclusion

Unlike traditional models of stand-alone software on a personal computer [6, 26–30], the web provides users from all over the world and on any computer

platform with the same materials located in centralized servers. Updating materials in online modules is greatly simplified with this centralization. With the widespread access to web resources, it is predicted that most medical educational and decision-support programs will find their way to the web in the near future [19–25].

Website URLs

CD-MarkerDX can be accessed at one the following web addresses: http://dpalm.med.uth.tmc.edu/faculty/bios/nguyen/nguyen.html http://dpalm.med.uth.tmc.edu/faculty/bios/nguyen/decision.html

In the event that these web addresses are changed due to some unexpected reason, please contact the authors by e-mail to obtain the new addresses: Nghia.D.Nguyen@uth.tmc.edu.

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Appendix: Listing of immunophenotype for each disorder [1–4]

1. Follicular small cleaved B-cell lymphoma
   (+) for: CD10, CD19, CD20, CD21, CD22, CD24, HLA-DR, sIg(−) for: CD2, CD3, CD4, CD5, CD7, CD8, CD11c, CD23, CD25, CD43, cIg,
2. Mantle B-cell lymphoma
   (+) for: CD5, CD19, CD20, CD22, CD24, CD43, HLA-DR, sIg, CD5/CD19 or CD5/CD20
   (−) for: CD11c, CD23, cIg
3. Large B-cell lymphoma
   (+) for: CD19, CD20, CD22, CD79a, HLA-DR,
   (−) for: CD2, CD3, CD5, CD7
4. Mediastinal B-cell lymphoma
   (+) for: CD19, CD20, CD22, CD79a, PC-1
   (−) for: CD2, CD3, CD5, CD7, CD10, CD15, CD21, sIg, TdT
5. B-cell lymphoma, unclassifiable, mixed small and large cells
   (+) for: CD19, CD20, CD22, CD79a, HLA-DR, sIg
   (−) for: CD2, CD3, CD5, CD7
6. Lymphoplasmacytoid B-cell lymphoma
   (+) for: CD19, CD20, CD22, CD79a, sIg, cIg
   (−) for: CD5, CD10, CD23
7. Marginal B-cell lymphoma
   (+) for: CD19, CD20, CD22, CD79a, sIg
   (−) for: CD5, CD10, CD23

8. Burkitt's B-cell lymphoma/ Acute lymphoblastic B-cell leukaemia, L3
    (+) for: CD10, CD19, CD20, CD22, CD77, CD79a, HLA-DR, sIg
    (−) for: CD5, CD23, TdT
9. Splenic B-cell lymphoma with villous lymphocytes
    (+) for: CD19, CD20, CD22, CD79a, HLA-DR, sIg
    (−) for: CD5, CD10, CD23, CD25
10. Peripheral T cell lymphoma
    (+) for: CD2, CD3, CD5, CD7
    (−) for: CD19, CD20, CD22, Cytokeratin
11. Lymphoblastic lymphoma (T cell)
    (+) for: CD2, CD3, CD5, CD7, CD38, CD71, TdT
    (−) for: CD15, CD20, Cytokeratin
12. Thymoma
    (+) for: TdT, Cytokeratin, CD4, CD8
    (−) for: CD45
13. Chronic lymphocytic leukaemia (B cell)/Small lymphocytic lymphoma
    (+) for: CD5, CD19, CD20, CD21, CD23, CD24, CD43, CD79a, HLA-DR, sIg, CD5/CD19 or CD5/CD20
    (−) for: CD2, CD3, CD4, CD7, CD8, CD10, CD25, FMC-7
14. Chronic lymphocytic leukaemia (T cell)
    (+) for: CD2, CD3, CD5, CD7
    (−) for: CD16, CD25, CD56, CD57, TdT
15. Prolymphocytic leukaemia (B cell)
    (+) for: CD19, CD20, CD22, HLA-DR, sIg, FMC-7
    (−) for: CD2, CD3, CD4, CD7, CD8, CD10, CD11c, CD25, TdT
16. Hairy B-cell leukaemia
    (+) for: CD11c, CD19, CD20, CD22, CD25, CD79a, CD103, sIg, FMC-7
    (−) for: CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD23,
17. Sezary syndrome/ Mycosis fungoides
    (+) for: CD2, CD3, CD4, CD5
    (−) for: CD1, CD7, CD8, CD10, CD11c, CD16, CD19, CD20, CD22, CD25, CD56, CD57, sIg, TdT, Cytokeratin
18. Adult T cell leukaemia/ lymphoma
    (+) for: CD2, CD3, CD4, CD5, CD25, CD38, CD71, HLA-DR
    (−) for: CD1, CD7, CD8, CD10, CD11c, CD16, CD19, CD20, CD22, CD56, CD57, sIg, TdT, Cytokeratin
19. Acute lymphoblastic leukaemia (T cell precursor)
    (+) for: CD3, CD7, TdT
    (−) for: CD10, CD19, CD20, CD22, HLA-DR, Cytokeratin
20. Acute myeloblastic leukaemia without maturation, M1
    (+) for: CD13, CD33, HLA-DR
    (−) for: CD2, CD3, CD5, CD7, CD11b, CD14, CD15, CD41, CD42, CD61, CD71, sIg, Glycophorin A
21. Acute myeloblastic leukaemia with maturation, M2
    (+) for: CD13, CD15, CD33, HLA-DR
    (−) for: CD2, CD3, CD5, CD7, CD11b, CD14, CD41, CD42, CD61, CD71, sIg, Glycophorin A

22. Acute promyelocytic leukaemia, M3
    (+) for: CD13, CD15, CD33
    (−) for: CD2, CD3, CD5, CD7, CD11b, CD14, CD41, CD42, CD61, CD71, HLA-DR, sIg, Glycophorin A
23. Acute myelomonocytic leukaemia, M4
    (+) for: CD11b, CD13, CD14, CD15, CD33, HLA-DR
    (−) for: CD2, CD3, CD5, CD7, CD41, CD42, CD61, CD71, sIg, Glycophorin A
24. Acute monoblastic leukaemia, M5
    (+) for: CD11b, CD11c, CD13, CD14, CD15, CD33, HLA-DR
    (−) for: CD2, CD3, CD5, CD7, CD41, CD42, CD61, CD71, sIg, Glycophorin A
25. Acute erythroleukaemia, M6
    (+) for: CD71, Glycophorin A
    (−) for: CD2, CD3, CD5, CD7, CD11b, CD14, CD41, CD42, CD61, sIg
26 Acute megakaryoblastic leukaemia, M7
    (+) for: CD33, CD41, CD42, CD61
    (−) for: CD2, CD3, CD5, CD7, CD11b, CD13, CD14, CD15, CD71, sIg, Glycophorin A
27. Biphenotypic acute leukaemia, AML & T cell ALL
    (+) for: CD3, CD7, CD13, CD33, TdT
    (−) for: CD10, CD19, CD20, CD22, Cytokeratin
28. Biphenotypic acute leukaemia, AML & B cell precursor ALL
    (+) for: CD13, CD19, CD22, CD33, CD79a, HLA-DR, TdT
    (−) for: CD3, CD7, sIg
29. Large granular lymphocyte leukaemia, NK cell
    (+) for: CD2, CD5, CD16, CD56, HLA-DR
    (−) for: CD3, CD4
30. Large granular lymphoproliferative disorder, T cell
    (+) for: CD2, CD3, CD8, CD16, CD56, CD57
    (−) for: CD25, TdT
31. Acute lymphoblastic leukaemia (Early-B precursor)
    (+) for: CD19, CD22, CD79a, HLA-DR, TdT
    (−) for: CD3, CD4, CD5, CD7, CD8, CD10, sIg, cIg
32. Acute lymphoblastic leukaemia (CALLA)
    (+) for: CD10, CD19, CD22, CD79a, HLA-DR, TdT
    (−) for: CD3, CD4, CD5, CD7, CD8, sIg, cIg
33. Acute lymphoblastic leukaemia (Pre-B)
    (+) for: CD10, CD19, CD22, CD79a, HLA-DR, cIg, TdT
    (−) for: CD3, CD4, CD5, CD7, CD8, sIg