A Rule-based Expert System for Laboratory Diagnosis of Hemoglobin Disorders

Andy N. D. Nguyen, MD; Elizabeth A. Hartwell, MD; John D. Milam, MD

• ***Objective.***—**To illustrate the utility of a rule-based expert system in diagnosing hemoglobin disorders.**

Design.—**A rule-based expert system was developed for diagnosing hemoglobin disorders. This expert system runs on IBM-compatible personal computers and uses a backward-chaining search strategy to draw conclusions. Laboratory data (ie, results of hemoglobin electrophoresis, quantitative measurements of hemoglobin F and hemoglobin A2 levels, and result of a sickle cell screen) are processed by the system using defined rules to obtain a set of differential diagnoses. Additional data, such as hematologic parameters, ethnicity of the patient, and the presence or absence of certain clinical signs and symptoms, aid in making a final diagnosis. The rules in the current version of this expert system include diagnostic criteria for 71 hemoglobin disorders.**

Setting.—**Regional academic medical center.**

Patients.—**We tested the system by using 58 survey sample cases offered by the College of American Pathologists during the period of January 1989 through December 1994.**

Main Outcome Measure.—**The established diagnosis for a given case must be included in the list of differential diagnoses suggested by the expert system.**

Results.—**The expert system included the actual diagnosis as one of the top four differential diagnoses in 90% of the cases, whereas all the laboratories participating in the survey included it in 84% (mean) of the cases.**

Conclusion.—**We propose that this user-friendly expert system is a potential tool for computer-assisted diagnosis of hemoglobin disorders.**

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The detection and identification of abnormal hemoglobins is of major clinical importance. Numerous hemoglobin disorders are known, the clinical manifestations of which range from lethal to asymptomatic.[1] During the past decade, major advances in medical laboratory techniques have allowed clinical pathologists to effectively diagnose various hemoglobin disorders.[2–5] The patterns of hemoglobin bands obtained with alkaline and acid hemoglobin electrophoresis on agarose gels, together with the results of other supplemental tests and clinical data, help confirm the identity of many hemoglobin disorders. Since several hemoglobin disorders demonstrate similar laboratory and clinical findings, their diagnosis, especially that of the uncommon types, often poses a challenge to pathologists who interpret the results of hemoglobin electrophoresis. For this reason, the most efficient diagnostic sequence of tests is not always followed in complicated cases and can result in misdiagnosis.

The differential diagnosis of hemoglobin disorders involves pattern recognition of different disease variants that have very similar laboratory findings. Each hemoglobin disorder has a set of diagnostic criteria that consists of many components that are difficult to remember. This is especially true for the uncommon disorders. The criteria include the presence of bands on alkaline and acid pH gels, the percentage of hemoglobin contained in each band, a sickle cell screen test, the quantitative measurements of hemoglobin F (HbF) and hemoglobin A_2 (HbA_2), the ethnicity of the patient, peripheral blood indices and smear, and other laboratory tests. Since many of these criteria are easily defined in terms of if-then rules, a rule-based expert system is a logical approach for aiding the diagnosis of these disorders. In addition, a system that readily displays specific rules would be extremely convenient for pathologists who infrequently encounter the more uncommon hemoglobin disorders.

Expert systems have been applied to many problems encountered in laboratory medicine.[6–20] The development of rule-based expert systems has been greatly simplified with the introduction of expert system shells that provide an integrated set of tools for the creation of individualized expert systems.[21] Many expert system shells are currently available as off-the-shelf packages.[21] In this study, we used our own expert system shell, which had been developed previously, to achieve full flexibility in expert system design. Herein, we discuss disorders of hemoglobin synthesis as an overview and describe our development of an expert system for laboratory diagnosis of hemoglobin disorders, its validation process, and its potential role in laboratory diagnosis.

DISORDERS OF HEMOGLOBIN SYNTHESIS

Hemoglobin is a tetrameric protein composed of globin and four heme groups.[1] Globin is composed of two pairs of polypeptides, and each of the four polypeptides is associated with one heme group. Hemoglobin A (HbA) is the major normal adult hemoglobin and is composed of two α and two β chains in an $\alpha_2\beta_2$ tetramer. The two minor

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From the Department of Pathology and Laboratory Medicine, The University of Texas Health Science Center at Houston.

Reprint requests to Department of Pathology and Laboratory Medicine, The University of Texas Health Science Center at Houston, PO Box 20708, Houston, TX 77225 (Dr Nguyen).

adult hemoglobins are HbA_2 and HbF, in which the β chains are replaced by a δ chain and by a γ chain, respectively. HbF is the predominant hemoglobin in newborns, but within the first year of life, it is largely replaced by HbA. By adulthood, hemoglobins are present in the approximate proportions of 97% HbA, 2% HbA_2, and 1% HbF.

Disorders of hemoglobin synthesis can be divided into two types. The first type includes the hemoglobinopathies, which are caused by the formation of abnormal globin chains, resulting in an altered hemoglobin structure. The second type consists of the thalassemias, which are associated with deficient production of globin chains, resulting in a decreased amount of normal hemoglobin.[1] Combinations of these two types of disorders also occur. The term *hemoglobin disorders,* as used in this article, specifically refers to hemoglobinopathies and thalassemias.

Evaluation of the hemoglobin disorders usually consists of the following laboratory tests[2–5]: hemoglobin electrophoresis, a sickle cell screen test (solubility test), quantitative measurements of HbA_2 and HbF levels, a complete blood cell count, and a peripheral blood smear.

EXPERT SYSTEMS

An expert system is computer software that employs knowledge captured in a computer program to solve difficult problems that usually require human expertise. Well-designed expert systems imitate the reasoning process experts use to solve specific problems and can be used by nonexperts to improve their problem-solving capabilities and by experts as knowledgeable assistants. Expert systems also can maintain scarce knowledge resources and produce consistent results.

Expert systems were first developed by computer scientists in the mid-1960s. This period of research on artificial intelligence was dominated by a belief that the coupling of powerful computers and reasoning laws would enable superhuman performance in solving any problem.[22] Many computer programs generated with such high expectations, however, did not fulfill their inventors' dreams.

The evolution from general-purpose to special-purpose expert systems occurred with the development of DENDRAL by Feigenbaum at Stanford University.[23] DENDRAL was designed to infer the molecular structures of unknown compounds from mass spectral and nuclear magnetic response data. This system used a procedural code to systematically enumerate all the possible molecular structures and then used chemical data to prune this list to a manageable size.

DENDRAL was followed by MYCIN, a rule-based system developed by Shortliffe, also at Stanford University.[23] MYCIN was designed to assist clinicians in diagnosing bacterial infections for patient management during the critical 24- to 48-hour period after symptoms manifested. Early diagnosis and treatment of bacterial infections are critical and must be accomplished during a time when decision making is often imprecise because the relevant information from laboratory studies is not yet available.

Both DENDRAL and MYCIN performed well, at least at the level of human experts. Since the mid-1970s, many expert systems have found commercial applications. Notable systems include the following.

XCON (Digital Equipment Corporation, Maynard, Mass).—This rule-based system incorporated several thousand rules into the configuration of minicomputer systems sold by Digital Equipment Corporation.[24] It could configure complex systems in less than 1 minute with 95% accuracy, a task that required experienced configuration personnel about 30 minutes with an accuracy rate of only 65%. By 1985, all the VAX microcomputer systems were configured by XCON.

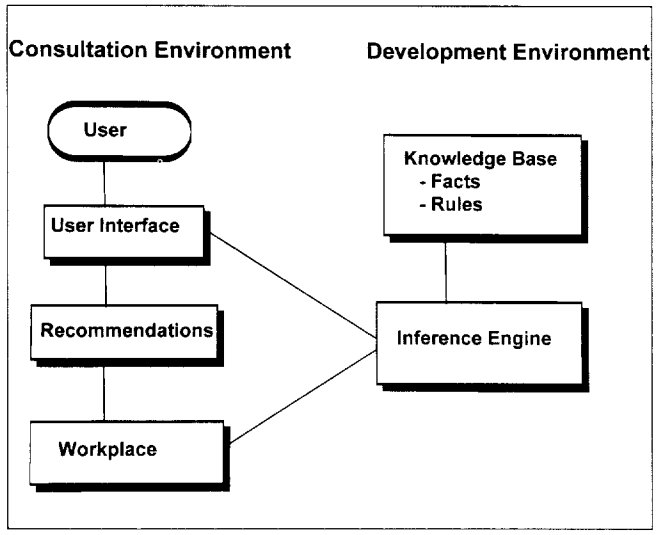

Fig 1.—*Structure of an expert system.*

DELTA (General Electric, Erie, Pa).—Built in 1980 by General Electric to troubleshoot diesel electric locomotive engines,[25] DELTA could uncover a faulty component within a few minutes and guide the locomotive engineer through the repair procedures. General Electric has now installed this system at every railroad repair shop.

Expert systems have two major components: the development environment and the consultation (runtime) environment (Fig 1).[21] The development environment is used by the expert system builder to construct the components and to enter information into the knowledge base. The consultation environment is accessed by the user to obtain expert knowledge and advice. The following components are seen in our expert system.

Knowledge Base.—This component contains the knowledge required for formulating and solving problems. It contains facts in the domain area and rules that direct the use of facts to diagnose specific disorders. The diagnostic criteria are the facts, also known as attributes, that are necessary to confirm a certain disorder. Potential sources of knowledge include human experts, textbooks, and databases.

Blackboard (Workplace).—This component is an area of working memory for the description of a current problem, as specified by the input data. The blackboard also records intermediate hypotheses and conclusions.

Inference Engine.—The brain of the expert system, this component provides the methodology for reasoning by using information in the knowledge base and in the blackboard to formulate conclusions.

User Interface.—This component allows communication between the user and the expert system. The user uses this interface to input data (positive attributes found in a patient) and also to obtain the results. This communication interface is usually a graphics format for ease of use (graphical user interface).

Table 1.—List of 71 Hemoglobin Disorders in XPHEMO Knowledge Base*

Normal Hb	HbQ (Thailand)/H
HbA_2' (homozygous)	HbS (sickle cell) disease
Hb British Columbia trait	HbS (sickle cell) trait
HbC disease	HbSC disease
HbC trait	HbS/C Harlem
HbC Harlem (homozygous)	HbSD disease
HbC Harlem trait	HbSE
HbC/C Harlem	HbSG
HbCE	HbS/Hope
HbCG	HbSN
HbCO	HbSO
HbC/β^0-thalassemia	HbS/α-thalassemia
HbC/β^+-thalassemia	HbS/β^0-thalassemia
Hb Chesapeake trait	HbS/β^+-thalassemia
HbD disease	Hb Zurich trait
HbD (Punjab, Los Angeles) trait	HbH disease
HbDC	Acquired HbH disease
HbDE	HbH/Constant Spring
HbDO	(α-thalassemia/Constant Spring)
HbE disease	α-Thalassemia minor
HbE trait	β^0-Thalassemia major
HbE/α-thalassemia	β^+-Thalassemia major
HbE/β^0-thalassemia	β-Thalassemia minor
HbE/H (Constant Spring type)	$\delta\beta$-Thalassemia (homozygous)
Hb Fort Worth trait	$\delta\beta$-Thalassemia (heterozygous)
HbG disease	Hb Constant Spring (homozygous)
HbG (Coushatta) trait	H Constant Spring (heterozygous)
HbG (Philadelphia) trait	HPFH (homozygous)
Hb Hasharon trait	HPFH (heterozygous)
Hbl trait	HPFH/C
Hbl trait	HPFH/S
Hb Kempsey trait	HPFH/β^+-thalassemia
Hb Koln	Hb Lepore (homozygous)
HbO trait	Hb Lepore (heterozygous)
HbQ (Thailand) trait	Hb Lepore/A_2'
HbQ/α-thalassemia 2	Hb Lepore/β^+-thalassemia

*Hb indicates hemoglobin; HPFH, hereditary persistence of fetal hemoglobin.

MATERIALS AND METHODS

Our study was performed in two phases. The first phase involved development of the expert system. In the second phase, the system was validated by using previously interpreted survey cases of hemoglobin disorders provided by the College of American Pathologists (CAP). Before beginning this project, we developed an expert system shell, XP (for eXPert), for general applications. This shell was written in Microsoft Visual C++ (Microsoft Corp, Redmond, Wash) language and was run on a Microsoft Windows 3.1 platform on an IBM-compatible computer. This shell has also been ported to other software platforms, including Borland C++ (Borland, Santa Cruz, Calif) and ANSI C, and other operating systems, including UNIX and Microsoft DOS. The XP shell is rule-based, and its inference engine uses a backward-chaining search strategy to draw conclusions.[26]

Expert System XPHEMO

Expert system XPHEMO was developed by applying the XP shell to the domain of hemoglobin disorders. A total of 71 different hemoglobin disorders were included in the knowledge base of this expert system (Table 1). The diagnostic criteria for hemoglobin disorders were based on the following attributes: (1) presence of electrophoretic bands on the alkaline pH gel with control hemoglobins C, S, F, or A; (2) presence of electrophoretic bands on the acidic pH gel with control hemoglobins C, S, F, or A; (3) densitometric measurements of the bands on the alkaline pH gel; (4) quantitative measurement of the HbF level, usually performed by using radial immunodiffusion; (5) quantitative measurement of the HbA_2 level, usually performed by using column chromatography; (6) screen test for sickle hemoglobin; and (7) clinical data and other laboratory tests, such as a peripheral blood smear, red blood cell indices, an isopropanol test, and a heat stability test.

A total of 111 attributes were included in the XPHEMO knowledge base (Table 2). Figure 2 shows the system's graphic user interface for entering data and obtaining the suggested interpretations. Many different hemoglobin disorders demonstrate the same electrophoretic mobilities on alkaline or acidic pH gels. For each designated location on the electrophoretic gels, which corresponds to the migration patterns of the control hemoglobins C, S, F, and A, the presence or absence of a band and its associated densitometric measurement are entered as data for the patient sample. For example, HbD is expected to produce a band at the approximate location of the control HbS band on an alkaline gel and a band at the approximate location of the control HbA on an acidic gel. Consequently, the input data should include an alkaline band at the HbS location and an acid band at the HbA location. Bands that are located nearer to the cathode than the HbC band (such as Hb Constant Spring and HbA_2') or nearer to the anode than the HbA band (such as Hb Bart's and HbH) on the alkaline gel should also be specified as such as shown in Fig 2. The same rule is applicable for a band located between HbA and HbF on the acidic gel (such as Hb Camden and Hb Hope).

The input of six data items (items 1–6 in the list above) is requested by XPHEMO. The unavailability of any of these data does not prevent XPHEMO from processing the data; however, incomplete data may compromise the accuracy of the differential diagnoses, that is, derive differential diagnoses with low certainty factors. The certainty factor for a disorder reflects how well its attributes match the data in a given case. This factor is defined as:

$$CF_i = \Sigma\ \alpha_{ij} A_{ij}$$

Table 2.—List of 111 Attributes in XPHEMO Knowledge Base

1. Presence of band in alkaline gel location: A_2'	57. Jaundice (high total bilirubin)
2. Presence of band in alkaline gel location: carbonic anhydrase	58. Asymptomatic or benign course
3. Presence of band in alkaline gel location: CoSp	59. A_2(Col Chro) < 1
4. Presence of band in alkaline gel location: C	60. 1 < A_2(Col Chro) < 6
5. Presence of band in alkaline gel location: S	61. 3.5 < A_2(Col Chro) < 15
6. Presence of band in alkaline gel location: F	62. 1 < A_2(Col Chro) < 3.5
7. Presence of band in alkaline gel location: A	63. 1 < CoSp < 2
8. Presence of band in alkaline gel location: J	64. 4 < CoSp < 10
9. Presence of band in alkaline gel location: H	65. 10 < C < 30
10. Presence of band in acid gel location: C	66. 30 < C < 45
11. Presence of band in acid gel location: S	67. 45 < C < 60
12. Presence of band in acid gel location: A	68. 60 < C < 85
13. Presence of band in acid gel location: Hope	69. 85 < C < 100
14. Presence of band in acid gel location: F	70. 15 < C < 95
15. Absence of band in alkaline gel location: A_2'	71. C > 95
16. Absence of band in alkaline gel location: carbonic anhydrase	72. 20 < C < 40
17. Absence of band in alkaline gel location: CoSp	73. 5 < S < 7
18. Absence of band in alkaline gel location: C	74. 5 < S < 15
19. Absence of band in alkaline gel location: S	75. 15 < S < 35
20. Absence of band in alkaline gel location: F	76. 25 < S < 45
21. Absence of band in alkaline gel location: A	77. 45 < S < 60
22. Absence of band in alkaline gel location: J	78. 60 < S < 60
23. Absence of band in alkaline gel location: H	79. 75 < S < 90
24. Absence of band in acid gel location: C	80. 80 < S < 100
25. Absence of band in acid gel location: S	81. S > 50
26. Absence of band in acid gel location: A	82. S > 90
27. Absence of band in acid gel location: Hope	83. S = 0
28. Absence of band in acid gel location: F	84. 5 < A < 35
29. African ancestry	85. 15 < A < 30
30. Asian ancestry	86. 30 < A < 50
31. Mediterranean ancestry	87. 50 < A < 70
32. Middle Eastern ancestry	88. 65 < A < 85
33. Native American ancestry	89. 80 < A < 92
34. North European ancestry	90. 90 < A < 95
35. Religion: Jewish	91. 95 < A < 100
36. Microcytosis	92. 40 < A < 60
37. Hypochromic	93. 70 < A < 95
38. Anemia	94. 40 < J < 60
39. Anisopoikilocytosis	95. 15 < J < 35
40. Reticulocytosis	96. 30 < H < 70
41. Presence of target cells	97. 3 < H < 30
42. Presence of nucleated RBCs	98. 0 < F(RID) < 7
43. Presence of sickle cells	99. 1 < F(RID) < 20
44. Rhomboidal intraerythrocytic inclusion	100. 5 < F(RID) < 10
45. Basophilic stipling of RBCs	101. 15 < F(RID) < 40
46. Presence of Heinz bodies	102. 60 < F(RID) < 95
47. Erythrocytosis	103. 95 < F(RID) < 100
48. Kleihauer-Betke test: stain evenly distributed in RBCs	104. F(RID) > 60
49. Kleihauer-Betke test: stain *not* evenly distributed in RBCs	105. 5 < F(RID) < 85
50. Solubility (or sickling) test: positive	106. 0.5 < F(RID) < 3
51. Solubility (or sickling) test: negative	107. 8 < F(RID) < 15
52. Isopropanol denaturation test: positive	108. 15 < F(Dens) < 35
53. Isopropanol denaturation test: negative	109. 50 < F(Dens) < 70
54. Heat instability test: positive	110. 35 < F(Dens) < 50
55. Heat instability test: negative	111. 80 < F(Dens) < 100
56. Splenomegaly	

* RBCs indicates red blood cells; Col Chro, column chromatography; RID, radial immunodiffusion; Dens, densitometry.

Where:

CF_i = Certainty factor for the ith disorder.
α_{ij} = Weight of the jth attribute in diagnosing the ith disorder ($0 < \alpha_{ij} < 1$, $\Sigma \alpha_{ij} = 1$).
A_{ij} = 1 if the jth attribute is present (positive data).
A_{ij} = 0 if the jth attribute is not present (negative data).

Each attribute is assigned a weight (ranging from 0 to 1) that is a measure of its relative importance in diagnosing a hemoglobin disorder. To demonstrate how the knowledge base of the expert system XPHEMO is implemented, the diagnostic criteria for sickle cell disease (HbSS) are given below as an example:

1. Presence of bands at the locations of HbS and HbF on the alkaline pH gel (weight = 0.08 for each band).
2. Presence of bands at the locations of HbS and HbF on the acid pH gel (weight = 0.08 for each band).
3. The percentage of HbS on the alkaline pH gel is more than 80% by densitometric measurement (weight = 0.11).
4. The quantitative measurement of HbF by the radial immunodiffusion method is from 1% to 20% (weight = 0.11).
5. The quantitative measurement of HbA_2 is from 2.0% to 4.5% by the column chromatography method (weight = 0.11).
6. The sickle cell screen test is positive (weight = 0.03).
7. Other laboratory data and clinical information needed to confirm this diagnosis include African ancestry; anemia; presence of sickle cells, nucleated red blood cells, and target cells in a peripheral blood smear; and elevated bilirubin (weight = 0.33).

A summary of the diagnostic criteria for sickle cell disease is

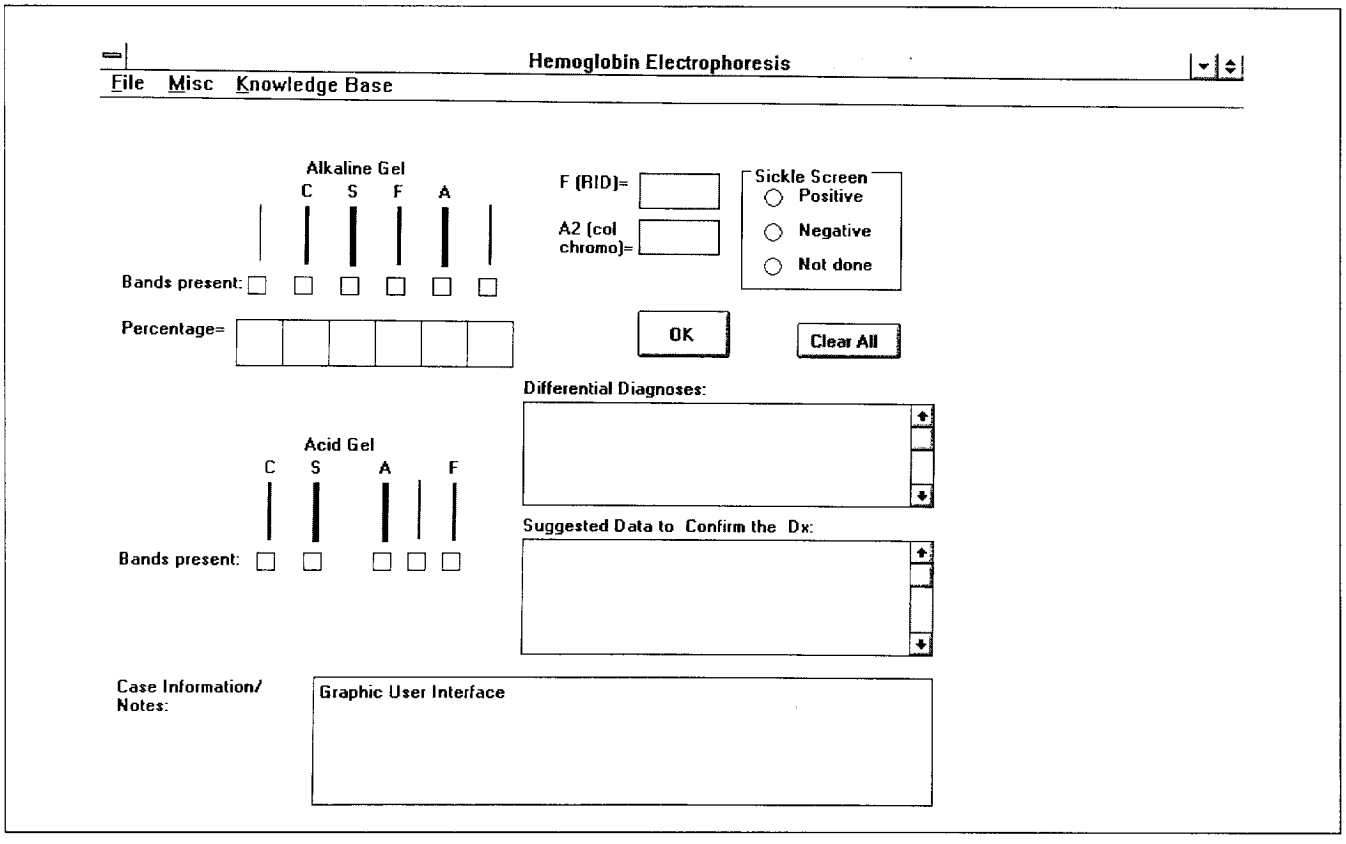

Fig 2.—*Graphic user interface to enter data and obtain suggestions.*

shown in Fig 3. The diagnostic weights of items 1 through 7 in the criteria are distributed as follows: items 1 and 2 (presence of bands on gels) = ⅓; items 3, 4, 5, and 6 (quantitative data on hemoglobins) = ⅓; and item 7 (clinical data and confirmatory tests) = ⅓.

The weight assigned to each group is divided equally among each of the components; for example, the weight of ⅓ for items 1 and 2 is divided into four equal parts of 0.08 for the presence of each band on the gels. Data may be entered only for items 1 through 6. This limitation is based on the assumption that these are the only data available to the user at the time of laboratory interpretation for most clinical cases. Item 7 involves clinical information and other confirmatory tests that may not be available at the time the electrophoretic gels are interpreted. Items 1 through 6 will be processed by the inference engine of the expert system, and a list of several differential diagnoses will be displayed. These diagnoses will be listed with their associated certainty factors. Since only items 1 through 6 are entered by the user, the maximum certainty factor that may be achieved is ⅔ (about 67%). The remaining ⅓ (33%) of the certainty factor may also be achieved by pursuing confirmatory data after consultation with XPHEMO (item 7). In addition, there are exclusion rules applied to the diagnostic criteria. These exclusion rules optimize the search process such that certain disorders are excluded early on if they do not meet the critical rules associated with the diagnosis. For example, sickle cell disease should be excluded from the search if any of the following data are entered: (1) the sickle cell screen test is negative; (2) bands are present at the locations of HbA or HbH (or both) on the alkaline gel; (3) a band is absent at the location of HbS in the alkaline gel; (4) bands are present at the locations of HbC or HbA (or both) on the acidic gel; or (5) a band is absent at the location of HbS on the acidic gel.

A demonstration of the typical consultation for a patient with sickle cell disease case is illustrative of how the expert system can be used and how its search mechanism works. Figure 4 shows the laboratory data available for a patient sample. XPHEMO tries to match this set of data with those that are characteristic of the 71 hemoglobin variants in the knowledge base. The data in this case match the following attributes for sickle cell disease: (1) bands present at the locations of HbS and HbF in the alkaline gel (weight = 0.08 for each band; total weight = 0.16); (2) bands present at the locations of HbS and HbF in the acidic gel (weight = 0.08 for each band; total weight = 0.16); and (3) a positive sickle cell screen test (weight = 0.03).

The certainty factor for sickle cell disease as a diagnosis at this point is the sum of all the weights for the attributes (above) that matched, the value being 0.35 (or 35%). After XPHEMO scanned all the 71 hemoglobin variants in the knowledge base, two other entities besides sickle cell disease were found to have a certainty factor value above the threshold level of 0.20 (20%): hereditary persistence of fetal hemoglobin (HPFH)/S, and HbS/β^0-thalassemia.

The threshold level may be set at any arbitrary value. For our project, a threshold of 0.20 seemed to work well for all the sample cases we tested. This level effectively limits the list of differential diagnoses to about three to four entities and clearly excludes those diagnoses that poorly match the given data. If the data input is inadequate, such that even the highest certainty factor (of the leading diagnosis) is below the threshold level, an error message will be generated to alert the user of this problem. Along with the list of differential diagnoses, a list of data suggested to confirm the most probable diagnosis (HPFH/S) will be generated (Fig 4). Note that HPFH/S has a slightly higher certainty factor than does sickle cell disease at this point. In fact, with the data given, these two diagnoses matched the case sample to almost the same degree. For comparison, the diagnostic criteria for HPFH/S are shown in Fig 5.

To see how the expert system accommodates additional data in the diagnostic process, let us assume that more data are available for the sample case: an HbF level of 3.2%, an HbA_2 level of

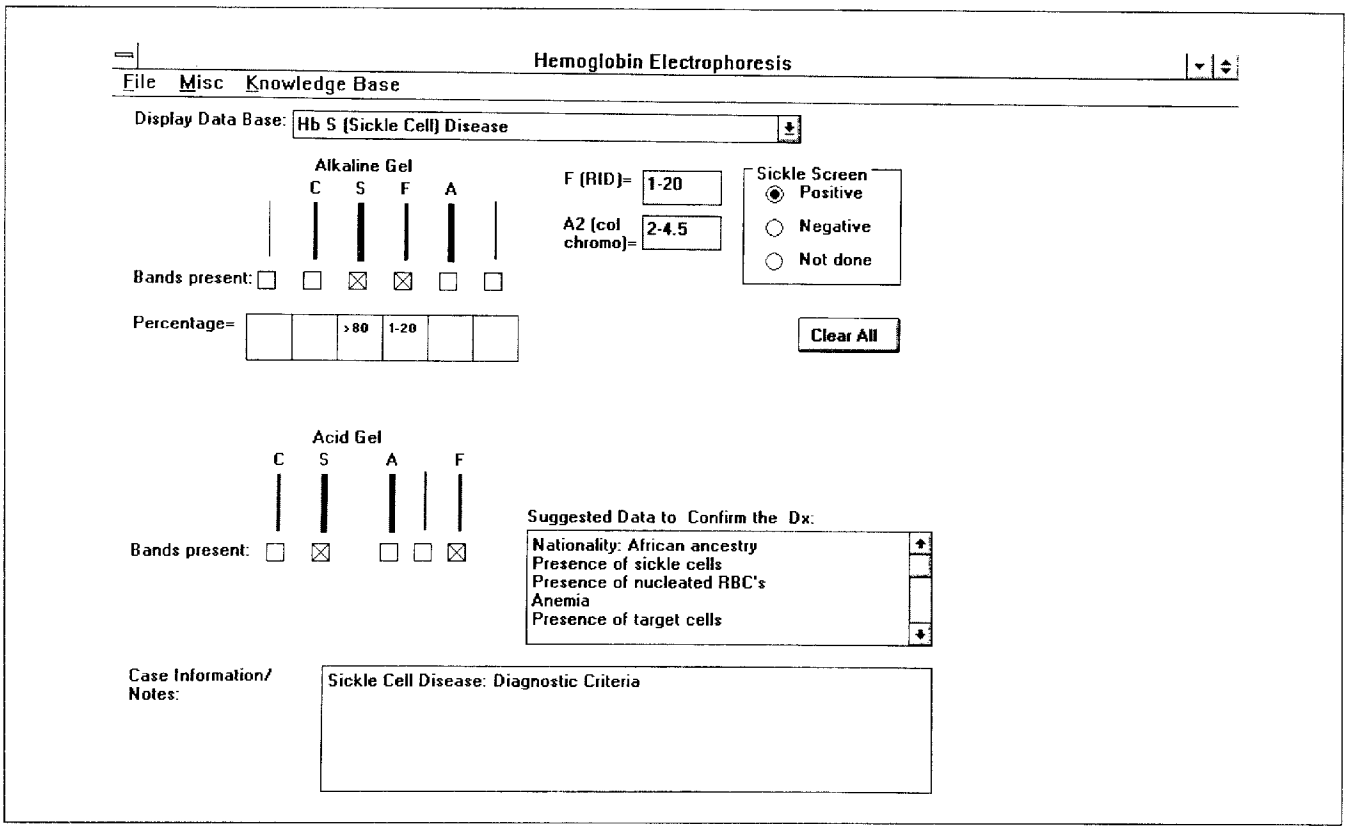

Fig 3.—*Summary of diagnostic criteria for sickle cell disease.*

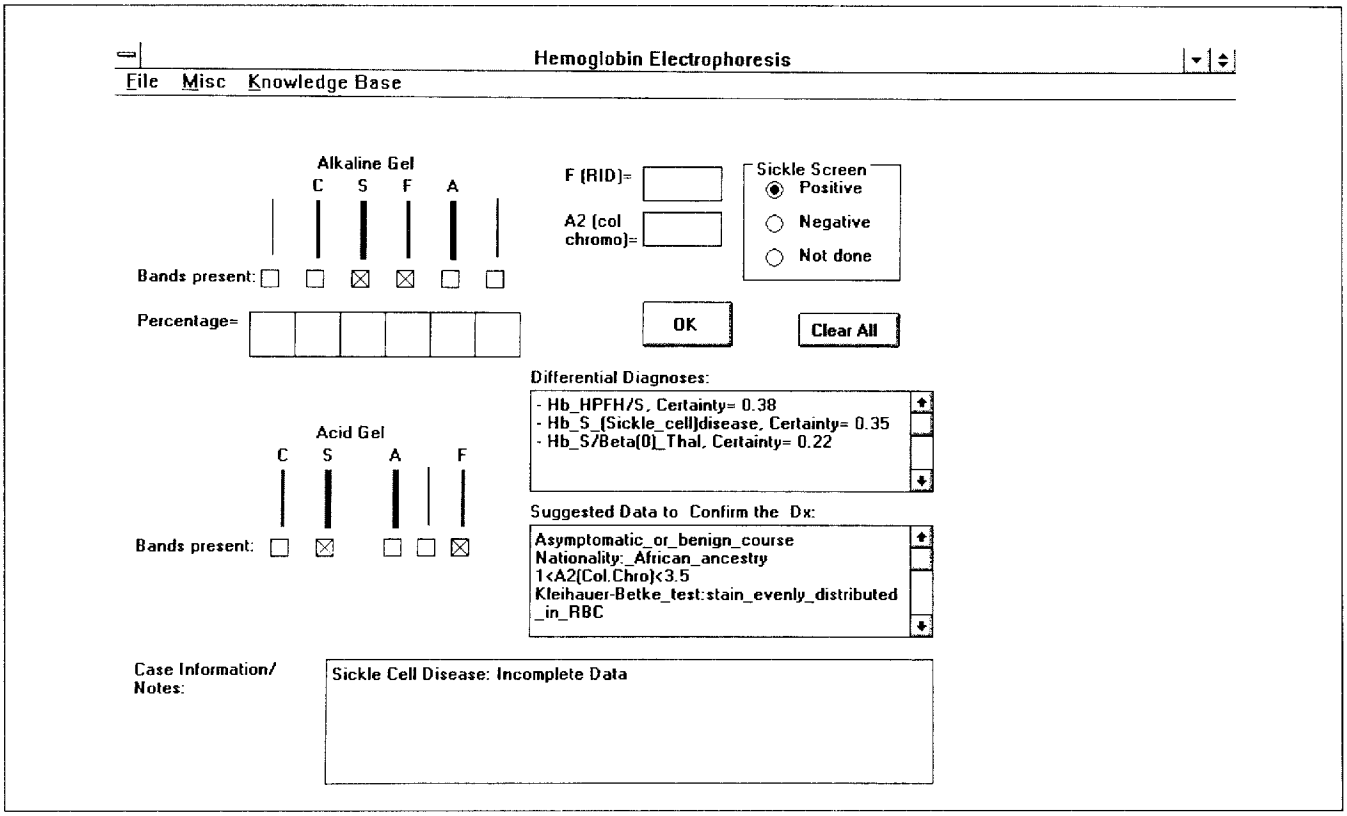

Fig 4.—*Demonstration of a sickle cell disease case with incomplete data input.*

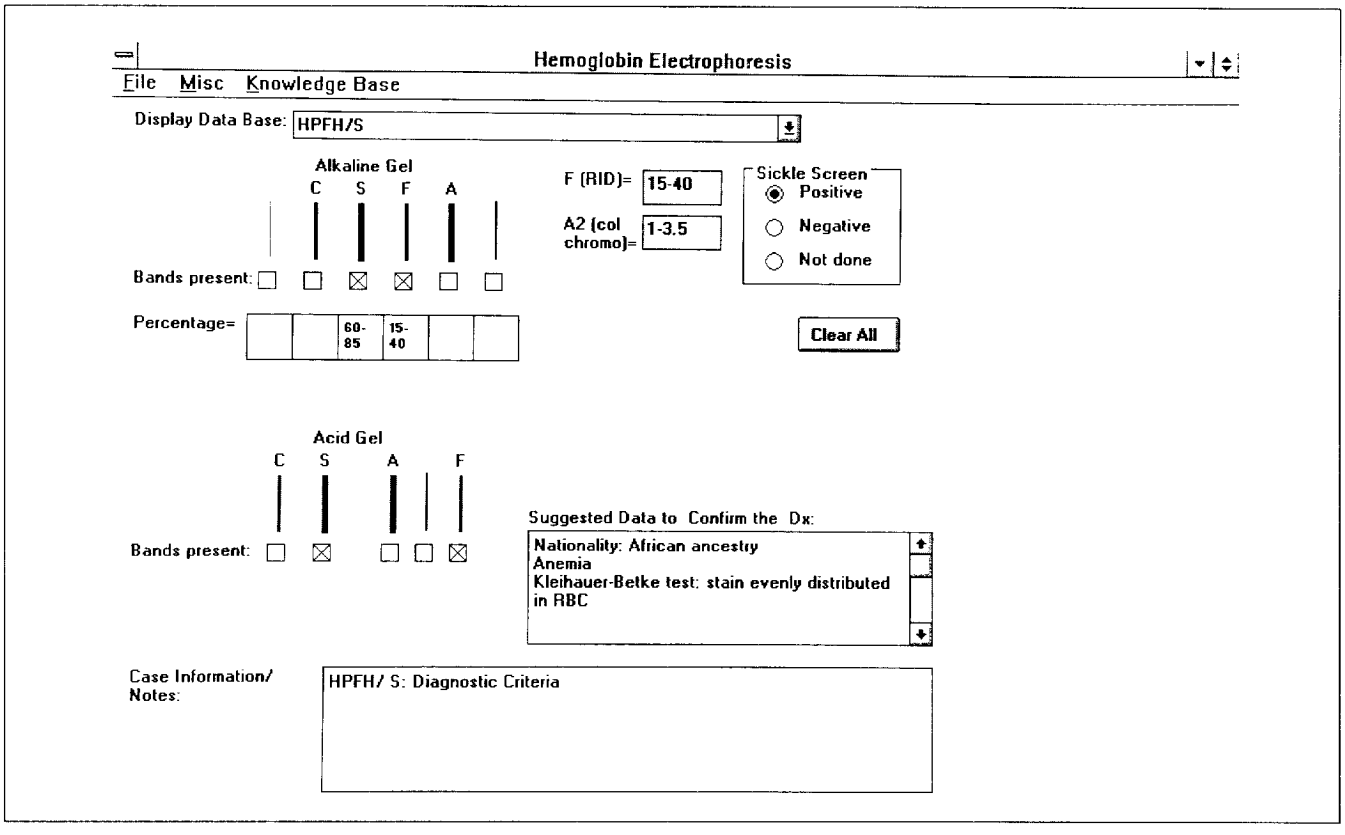

Fig 5.—*Diagnostic criteria for hereditary persistence of fetal hemoglobin/S.*

3%, and densitometric readings of 98% and 2% for the bands at the locations of HbS and HbF on the alkaline gel, respectively. These additional data were entered as shown in Fig 6. The expert system incorporated these new data into the search and assigned a higher certainty factor for sickle cell disease (0.68) than for HPFH/S (0.49). This change in the certainty factor occurred because the level of HbF is typically more than 15 in HPFH/S and is less than 20 in sickle cell disease. At this point in the analysis, with a certainty factor of 0.31, the diagnosis of HBS/β^0-thalassemia was unlikely.

Before a final diagnosis is made, all three differential diagnoses in the list should be considered. The diagnostic criteria for each differential diagnosis can be reviewed efficiently by using the "Display Disease Attributes" feature of XPHEMO. Sickle cell disease would be favored as a diagnosis if the following remaining attributes were identified: sickle cells and nucleated red blood cells in a peripheral blood smear. On the other hand, if HPFH/S were suspected, a Kleihauer-Betke test could be performed to check for the pattern of uniform staining of HbF in all the erythrocytes. The last component of this analysis demonstrated the critical role of the interpreter in finalizing the diagnosis. The expert system is only helpful in suggesting the differential diagnoses. The final diagnosis still must be established by the interpreting pathologist.

The inference engine of XPHEMO was implemented in Microsoft Visual C++ as part of the XP shell. The graphic user interface of XPHEMO was designed by using Microsoft Visual Basic. As shown in the preceding demonstration, XPHEMO was designed to be user-friendly. The graphical user interface was arranged such that the sequence of data entry, display of results, or review of the knowledge base would be intuitive because of the way the interface presents to the user. Besides the essential features that have been shown in the demonstration, XPHEMO has also been designed to include the following features.

1. "What If" reruns can be performed when the user modifies one or more of the data elements. This feature enhances the flexibility of XPHEMO in evaluating data that may not always be clear-cut, that is, when the locations of electrophoretic bands or the quantities of hemoglobins are borderline. Results from "What If" reruns would give the user the opportunity to select the most likely diagnosis when evaluating laboratory or clinical data that may be subject to errors due to various reasons.
2. Displaying the approximate locations of all the hemoglobin variants on an alkaline or acidic gel relative to the locations of the control hemoglobins C, S, F, and A for user information.
3. Printing the contents on the computer screen at any time during the session.
4. Displaying on-line "HELP" instructions for using XPHEMO.

Method for Validating Expert System XPHEMO

In the second phase of our project, we used 58 sample cases involving various hemoglobin disorders to test expert system XPHEMO. These were survey sample cases offered by the CAP.[27] All the sample cases used in this validation study covered the period from January 1989 to December 1994 and were tested retrospectively (Table 3). Not included in this validation study were cases that involved newborn patients or cases that were missing from our survey files. Data entry for each case included only the data that were available in the Immunology Laboratory at Hermann Hospital (Houston, Tex) at the time of original interpretation to ensure that the expert system would not possess more data in the retrospective study than was originally available when the survey sample cases were originally diagnosed. To avoid potential bias in the design of XPHEMO, its knowledge base had been developed by one of the authors who had not interpreted the CAP surveys previously.

Hemoglobin electrophoresis was performed by using Paragon hemoglobin electrophoresis kits (Beckman Paragon, Brea, Calif) and applying hemolysates to alkaline or acidic agarose gels. After electrophoresis, the hemoglobins were fixed in the gels by air drying. The alkaline films were stained with Paragon blue, and the acidic films were stained with Paragon violet. The hemoglo-

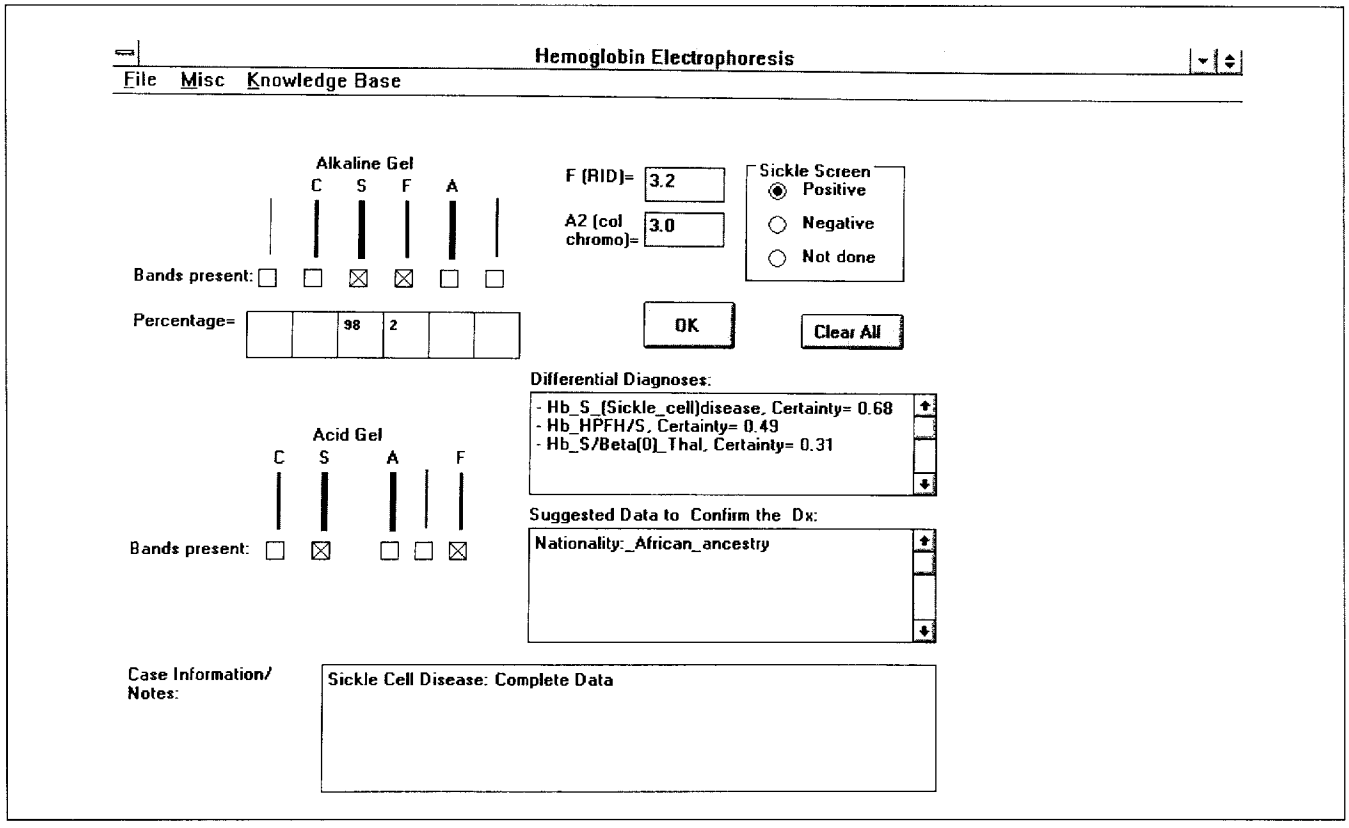

Fig 6.—*Demonstration of a sickle cell disease case with complete data input.*

bin pattern of each hemolysate was then compared to the hemoglobin standards (HbA, HbF, HbS, and HbC) on the same gels. Quantitative measurements of the hemoglobin bands were performed by densitometric scanning of the alkaline film at an optical density of 600 mm.

Fetal hemoglobin (HbF) was measured by using the radial immunodiffusion method (HbF QUIPlate, Helena Laboratories, Beaumont, Tex). Standards of known HbF concentrations and dilutions of the patient's hemoglobin were applied to the wells in the gel. The HbF antigen combined with the HbF antibody in the gel, producing an opaque precipitin ring around the wells. After 24 hours, the diameter of the precipitin ring was measured. The square of the diameter was proportional to the HbF concentration. A reference curve was prepared from the known concentrations of hemoglobin standards, and the HbF concentration of the patient sample was intrapolated from this curve.

Quantitative measurement of the HbA_2 level was performed by using Quick Column Microchromatography HbA_2 kits (Helena Laboratories). The Sickle-Thal column was used if HbS was present; the β-Thal column was used otherwise. In this anion-exchange chromatography, the buffer concentration and the pH value were controlled to produce varying negative charges in the different hemoglobins. These negatively charged hemoglobins bound to the positively charged cellulose resin. After binding, the hemoglobins were removed selectively from the resin by altering the pH of the solution buffer. The absorbance of each hemoglobin fraction was measured, and the A_2 fraction was calculated accordingly.

A solubility test for sickling hemoglobin was performed by using the Sickle-Sol Test Kit (Dade, Miami, Fla). A blood sample was added to a solution containing sodium hydrosulfite (to reduce the hemoglobin), a concentrated phosphate buffer, and saponin (to lyse the red blood cells). If the solution became turbid enough to obscure the reading of ruled black lines placed behind the test tube, the solubility test was positive.

For an XPHEMO session to be considered successful, the established diagnosis for a given case must have been included in the list of differential diagnoses suggested by XPHEMO at the end of the consultation session. The established diagnosis was the diagnosis given in the survey testing reports of the CAP.

RESULTS OF THE VALIDATION PROCESS

Table 3 shows all of the cases used in the validation process with the accompanying data: actual diagnosis, ranking of the actual diagnosis by XPHEMO (if the actual diagnosis was missed, the reasons for this), and the percentage of participating laboratories that gave a correct diagnosis in the surveys conducted by the CAP. Since each laboratory was allowed four differential diagnoses for each survey case, the performance of XPHEMO could be meaningfully compared with that of the participating laboratories. The final results, summarized in Table 4, show a success rate of 90%, that is, 90% of the actual diagnoses were included in the four most probable diagnoses gen-

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* Hb indicates hemoglobin; HPFH, hereditary persistence of fetal hemoglobin.
† Not ranked owing to incorrect value of HbF (technical error).
‡ Not ranked owing to incorrect value of HbA2 (technical error).
§ Not ranked owing to red blood cell transfusion or exchange.

Table 3.—Validation Results of 59 College of American Pathologists Survey Cases*

Case No.	Established Diagnosis	Ranking of Diagnosis by XPHEMO	Percentage of Laboratories With Correct Diagnosis
	1989		
1	HbS/β^+-Thalassemia	1	92
2	Hb Hasharon trait	1	100
3	HbQ/α-thalassemia 2	2	86
4	No abnormal Hb	Not ranked†	82
6	HbC/β^+-thalassemia	1	47
7	β-Thalassemia minor	2	84
8	HbE/α-thalassemia	2	100
9	No abnormal Hb	1	77.1
	1990		
1	HbS trait	1	84
2	HbA2′ (homozygous)	2	86
3	Hb British Columbia trait	1	84
5	HbD trait	2	100
6	HbS/Hope	1	43
7	HbH disease	3	98
8	HbS trait	1	84
9	HbC/β^0-thalassemia	2	100
10	Hbl trait	1	56.1
11	HbS trait	1	84
12	HbD trait	1	100
13	β-Thalassemia minor	1	96
	1991		
1	HbS trait	1	99
2	HbG trait	4	100
3	Hb Lepore (heterozygous)	2	77.1
4	Hbδ/β (heterozygous)	2	75.1
5	HbS/C	1	90
6	HbS/E	2	84.2
8	No abnormal Hb	1	63
9	HbS trait	1	95.6
10	HbS/G	1	81
11	HbS trait	1	99.2
12	HbD trait	2	100
13	HbD trait	2	100
	1992		
1	HbS trait	1	98
2	HbO trait	1	69
3	No abnormal Hb	Not ranked‡	68
4	No abnormal Hb	Not ranked‡	100
5	HbE disease	1	88.3
6	HbS/N	1	100
8	Sickle cell disease, transfusion	Not ranked§	64
9	HbS/C, transfusion	Not ranked§	89.7
10	Hb Lepore (heterozygous)	1	71
11	HbS trait	1	96.3
12	Hb Koln	1	83
13	Sickle cell disease	Not ranked†	99
14	HbS/C (Harlem)	2	100
	1993		
1	HbS trait	1	86
2	HbE trait	4	82
5	No abnormal Hb	1	64
6	HbS/D	1	18
7	β-Thalassemia minor	2	87.5
8	HPFH/β^+-thalassemia	2	95.7
9	Hb HPFH/C	1	100
10	HbS trait	1	96.5
	1994		
1	HbS trait	1	99.6
2	Hbl trait	1	51.9
3	Hb Zurich trait	1	70
4	No abnormal Hb	1	93.4
5	HbD trait	2	88.1
6	HbC trait	1	98.5

Table 4.—Summary of Validation Results			
Ranking by XPHEMO	No. of Cases	Percentage	Accumulated Percentage
First differential diagnosis	35	59.3	59.3
Second differential diagnosis	15	25.4	84.7
Third differential diagnosis	1	1.7	86.4
Fourth differential diagnosis	2	3.4	89.8
Not ranked*	6	10.2	100
Total	**59**	**100**	**100**

* No ranking of the diagnosis by XPHEMO was due to incorrect value of HbF (technical error) in two cases, incorrect value of HbA_2 (technical error) in two cases, and red blood cell transfusion or exchange in two cases.

erated by XPHEMO. In 59% of the cases, the actual diagnosis was ranked as the most probable diagnosis with the highest certainty factor. Of the actual diagnoses, 10% (six cases) were not included in the list of differential diagnoses for the following reasons.

Inadequate Data Input.—(1) Two cases had an incorrect value of HbA_2 because of technical error. No hemoglobin disorders were associated with these cases (1992 case 3 and 1992 case 4). α-Thalassemia minor was ranked as the leading diagnosis by XPHEMO owing to an elevated value of HbA_2. However, XPHEMO did demonstrate the inconsistency of data via its suggested data for confirmation. The suggested data (hypochromic microcytic anemia) were not consistent with the clinical data given in the surveys. (2) Two cases had an incorrect value of HbF because of technical error. No hemoglobin disorders were associated with the first cases (1989 case 4). XPHEMO ranked α-thalassemia minor as the leading diagnosis because of an elevated value of HbF. However, the suggested data for confirmation (hypochromic microcytic anemia) were not consistent with the clinical information. Sickle cell disease was the correct diagnosis for the second case (1992 case 13). No diagnosis was given by XPHEMO owing to a low value of HbF. A message was displayed to warn the user that the data may be incorrect or inadequate.

Correct diagnoses were obtained with XPHEMO after data were correctly input for the four preceding cases, yielding a success rate of 96%.

Limitation of XPHEMO Knowledge Base.—Two cases had blood samples collected for testing after red cell transfusion. The correct diagnosis for these two cases (1992 case 8 and 1992 case 9) was sickle cell disease status post–red-cell transfusion. Without a known history of transfusion, it is impossible to differentiate sickle cell disease with transfusion from sickle cell trait, which was the diagnosis suggested by XPHEMO.

By comparison, the laboratories that participated in the surveys conducted by the CAP had a mean success rate of 84%, that is, 84% of the laboratories listed the correct diagnosis as one of the four differential diagnoses on their answer sheet.

COMMENT

To date, the application of expert systems in medicine has been limited but, nonetheless, impressive.[6–20] The current level of computer technology can support the analysis of patient data in a well-defined domain by means of an expert system. We successfully developed and tested an expert system that can be used as a tool in diagnosing hemoglobin disorders. The design of XPHEMO permits the entry of incomplete laboratory data, which is characteristic of laboratory workup of hemoglobin disorders, and facilitates meaningful gathering of data. This approach can significantly reduce the amount of irrelevant data that the interpreting pathologist must gather before making a diagnosis. Consequently, XPHEMO could potentially improve laboratory efficiency and make medical expert systems more acceptable. Hopefully, it can serve as the prototype of more powerful tools that will be used by pathologists in the laboratory of the future. These tools will be extremely useful to laboratory personnel in independent study as well as in clinical interpretation.[6–20,28] We recently introduced XPHEMO to the staff of the Immunology Laboratory at Hermann Hospital as a computer-assisted tool for pathology residents in diagnosing difficult hemoglobin disorder cases. The feedback from the residents using this expert system for clinical cases has been very positive (A.N.D.N., unpublished data, 1995).

Despite the utility of XPHEMO, there are certain constraints inherent in its use.

1. The user must have a functional knowledge of hemoglobin disorders to be able to use XPHEMO effectively because this expert system 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 read the electrophoretic gels are critical in the diagnostic process. XPHEMO can generate a list of differential diagnoses in most cases if adequate data are input. The interpreting pathologist can then quickly compare the patient's laboratory data to the rules available from the XPHEMO display module and make the appropriate diagnosis. The example of sickle cell disease we gave above underscores the importance of human judgment in ruling out other hemoglobin disorders, such as HPFH/S and HbS/β^0-thalassemia. We believe that the experienced pathologist's clinical judgment and the information gathered by using the screening function of XPHEMO should yield an appropriate diagnosis.

2. The knowledge base in the current version of XPHEMO needs to be improved. More hemoglobin disorders must be added to the knowledge base to cover a wider spectrum of disorders. As pathologists gain more knowledge about hemoglobin disorders, the information they gather can be added to the XPHEMO knowledge base.

3. The current version of XPHEMO does not include a knowledge base of data for patients who are less than 1 year of age owing to the difficulty in creating diagnostic rules for elevated HbF in such patients. It also does not include patients with hemoglobin disorders who have recently received red cell transfusion, cases in which HbA may mask the baseline hemoglobin variant pattern. Furthermore, disorders that exhibit normal Hb electrophoretic patterns, such as pyruvate kinase deficiency and glucose-

6-phosphate dehydrogenase (G6PD) deficiency, are not included in the XPHEMO knowledge base.

4. The differential diagnoses suggested by XPHEMO are ranked according to their certainty factors, which strictly reflect the degree of matching between the data and the attributes of the disorders as specified by the rules. These certainty factors do not take into account the incidence rates of different disorders that may have similar laboratory findings. The comprehensive list of differential diagnoses offered by XPHEMO helps prevent the user from overlooking some uncommon hemoglobin disorders. Human judgment, however, is still the critical factor in finalizing the diagnosis.

5. XPHEMO may not be needed to diagnose common hemoglobin disorders, such as sickle cell trait, especially in a laboratory that routinely encounters such cases. It proved to be useful only in diagnosing the less common hemoglobin disorders.

6. XPHEMO is designed for laboratories that use gel electrophoresis for hemoglobin identification. It is not intended for laboratories that use isoelectric focusing or high-performance liquid chromatography to diagnose hemoglobin disorders.

The development of user-friendly computers and the rapidly increasing number of physicians with skills and interests in computer-based applications create an ideal climate for the use of expert systems for diagnostic purposes. The number of expert system applications in laboratory medicine is expected to increase. Laboratory clinicians are in an ideal position to exploit expert systems because they tend to be computer literate and have access to a large amount of patient data on laboratory computers.[29] The databases of laboratory information systems provide a tremendous resource of high-quality information for validating expert systems.[30] Ultimately, acceptance of expert systems by the medical community depends on the performance of current and future applications.[18] The successful implementation of expert systems in the laboratory environment will encourage this acceptance.

In conclusion, we found that XPHEMO provides a convenient, interactive tool to assist clinical personnel in diagnosing hemoglobin disorders. The number and complexity of hemoglobinopathies require that the differential diagnoses suggested by XPHEMO be reviewed before making a final diagnosis. A logical extension of XPHEMO would be to integrate the knowledge base into existing laboratory instrumentation, such as the densitometry reader. Data could then be retrieved directly from the instrument for use by the inference engine, thus simplifying user interaction.

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Note.—The Microsoft-Windows version of the expert system XPHEMO is available from the authors at a nominal fee of $10 to cover the cost of a computer diskette and postage. Users will be provided with software instructions and the necessary technical support. Special arrangements may also be made to obtain the UNIX-based version of this expert system. Please write Andy N.D. Nguyen, MD, at the Department of Pathology and Laboratory Medicine, The University of Texas Health Science Center, PO Box 20708, Houston, TX 77225.

References

1. Jandl JH. Abnormal hemoglobins and hemoglobinopathies. In: Jandl JH. *Blood: Textbook of Hematology.* Boston, Mass: Little Brown and Co; 1987:361–406.

2. Huisman TH, Jonxis JHP. *The Hemoglobinopathies: Techniques of Identification.* New York, NY: Marcel Dekker; 1977.

3. Fairbanks VF. *Hemoglobinopathies and Thalassemias: Laboratory Methods and Clinical Cases.* New York, NY: Brian C. Decker; 1980.

4. Schmidt RM, Brosious EM. *Basic Laboratory Methods of Hemoglobinopathy Detection.* Atlanta, Ga: Centers for Disease Control and Prevention; November 1984. HEW publication (CDC) 75–8266.

5. Schmidt RM, Rucknagel DL, Necheles TF. Comparison of methodologies for thalassemia screening by Hb A2 quantitation. *J Lab Clin Med.* 1975;86:5.

6. Ryan C. Cost reduction and QC software on a microbiology identification and susceptibility system. *Am Clin Lab.* May 1995:26.

7. Marquardt VC. Artificial intelligence and decision-support technology in the clinical laboratory. *Lab Med.* 1993;24:777–782.

8. Barnett GO, Cimino JJ, Hupp JA. DXplain: an evolving diagnostic decision-support system. *JAMA.* 1987;258:67–74.

9. Forsstrom J, Nuutila P, Irjala K. Using the ID3 algorithm to find discrepant diagnoses from laboratory databases of thyroid patients. *Med Decis Making.* 1991;11:171–175.

10. Shortliffe EH. Computer programs to support clinical decision making. *JAMA.* 1987;258:61–66.

11. Groth T, Moden H. A knowledge-based system for real-time quality control and fault diagnosis of multitest analyzers. *Comput Methods Programs Biomed.* 1991;34:175–190.

12. Tischler AS, Martin MR. Generation of surgical pathology report using a 5,000 word speech recognizer. *Am J Clin Pathol.* 1989;92(suppl 4, pt 1):s44–s47.

13. Tong DA. Weaning patients from mechanical ventilation: a knowledge-based system approach. *Comput Methods Programs Biomed.* 1991;35:267–278.

14. Autio K, Kari A, Tikka H. Integration of knowledge-based system and database for identification of disturbances in fluid and electrolyte balance. *Comput Methods Programs Biomed.* 1991;34:201–209.

15. Weiss SM, Kulikowski CA, Galen RS. Representing experience in a computer program: the serum protein diagnostic program. *J Clin Lab Automation.* 1983;3:383–387.

16. Shifman MA. FABHELP: a rule-based consultation program for FAB classification of acute myeloid leukemia and myelodysplastic syndromes. *Lab Med.* 1991;22:639–643.

17. O'Connor ML, McKinney T. The diagnosis of microcytic anemia by a rule-based expert system using VP-Expert. *Arch Pathol Lab Med.* 1989;113:985–988.

18. Spackman KA, Connelly DP. Knowledge-based systems in laboratory medicine and pathology. *Arch Pathol Lab Med.* 1987;111:116–119.

19. Bates JE, Bessman JD. Evaluation of BCDE, a microcomputer program to analyze automated blood counts and differentials. *Am J Clin Pathol.* 1987;88:314–323.

20. Blomberg DJ, Ladley JL, Fatty JM, Patrick EA. The use of an expert system in the clinical laboratory as an aid in the diagnosis of anemia. *Am J Clin Pathol.* 1987;87:608–613.

21. Turban E. *Expert systems and Applied Artificial Intelligence.* New York, NY: MacMillan Publishing Co; 1992:73–114, 453–480.

22. Newell A, Simon H. *Human Problem Solving.* Englewood Cliffs, NJ: Prentice-Hall; 1973.

23. Buchanan BG, Shortliffe EH, eds. *Rule-Based Expert Systems.* Reading, Mass: Addison-Wesley; 1984.

24. Sviokla JJ. An examination of the impact of expert systems on the firm: the case of XCON. *MIS Quarterly.* June 1990:27–34.

25. Bonissone PP, Johnson HE Jr. Expert system for diesel electric locomotive repair. *Human Systems Management.* 1985;4.

26. Chignell M, Parsaye K. *Expert Systems for Experts.* New York, NY: John Wiley and Sons; 1988: chap 7.

27. *Hemoglobinopathy Survey.* Northfield, Ill: College of American Pathologists; 1989–1994.

28. Siegel JD, Parrino TA. Computerized diagnosis: implications for clinical education. *Med Educ.* 1988;22(1):47–54.

29. Korpman RA. Using the computer to optimize human performance in health care delivery: the pathologist as medical information specialist. *Arch Pathol Lab Med.* 1987;111:637–645.

30. Connelly DP. Embedding expert systems in laboratory information systems. *Am J Clin Pathol.* 1990;94(suppl 1):7–14.