

**PREDICTING THE EFFECTIVENESS OF HYDROXYUREA IN
INDIVIDUAL SICKLE CELL ANEMIA PATIENTS**

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Abstract

Treatment with hydroxyurea (HU) partially alleviates disease symptoms in many patients with sickle cell anemia. The study described in this paper was undertaken to develop the ability to predict the response of sickle cell patients to HU therapy. We analyzed the effect of HU on the values of 23 parameters or characteristics of each of 83 patients. A Student T-test was used to confirm⁹ at the 0.001 level that treatment with HU increases the proportion of red blood cells containing fetal hemoglobin (HbF), and that HU also increases the average corpuscular volume (MCV) of the red blood cells. Correlation analysis failed to establish a statistically significant relationship between any of the 23 parameters and the magnitude of the HbF response. Linear regression analysis also failed to predict a patient's response to HU. On the other hand, artificial neural network (ANN) pattern recognition analysis of the 23 parameters predicts, with 86.6% accuracy, those patients that respond positively to HU and those that do not. Furthermore, we have found that the values of only 10 of the 23 parameters are sufficient to train ANNs to predict which patients will respond to HU. Based on analyses with ANNs, the most important parameters in predicting a patient's response to HU are the duration of treatment with HU, red blood cell size, white blood cell count, platelet count, mean cell volume, neutrophil count, patient weight, Senegal (SEN) haplotype, reticulocyte count, and patient gender. A trained ANN accurately predicted HU response for ~85% of the patients included in the analyses.

Introduction

ANN-based pattern recognition techniques have had great success in identifying patterns or complex relationships that man, unassisted by computers, is unable to perceive^{14,15,18,31,35,38,40}. ANN technology involves extensive training of a computer to enable it to distinguish a pattern from many patterns that closely resemble each other. It is often difficult to identify the features an ANN uses to classify patterns (e.g. a responder versus a non-responder). It may be the presence or absence of a particular feature that enables an ANN to classify its patterns. Experimenting to find an effective ANN architecture and algorithm is a process that requires considerable time on a powerful computer. But once an ANN is selected and trained, its ability to identify new data is nearly instantaneous and requires only a PC.

ANNs have begun to be used for many different purposes in medicine¹⁶⁻³⁵. We believe there is enormous potential for ANNs to be applied widely in data analysis including assisting in diagnoses and in analyzing the characteristics of individual patients to identify the most effective treatment. The research project described in this manuscript was designed to assist physicians in predicting the response to HU therapy in patients with sickle cell anemia.

Adult hemoglobin (HbA) is a tetrameric protein composed of two α -chains and two β -chains ($\alpha_2\beta_2$). Sickle cell anemia is an inherited disease in which the two β -chains of normal adult hemoglobin are replaced by two mutated chains each of which has a single nucleotide substitution (GAG \rightarrow GTG) in the genes encoding the β -chains of HbA¹. The abnormal β -chains (β^s) contain a hydrophobic valyl residue in place of a negatively-charged glutamyl residue at position 6. Deoxygenated hemoglobin of sickle cell patients (HbS or $\alpha_2\beta^s_2$) is less soluble than deoxygenated HbA. Under conditions where HbS is depleted of oxygen, HbS aggregates, causing the erythrocytes to deform into the sickle shape that tends to block capillaries, the characteristics of the disease²⁻⁴.

Treatment with HU alleviates the clinical course in many patients with sickle cell anemia⁹. The beneficial effect of HU is believed to depend on its ability to increase the expression of the γ chains of HbF ($\alpha_2\gamma_2$)^{10-11, 43}. Red blood cells that contain HbF are referred to as F-cells. Most patients respond to HU with an increase in the HbF concentration of blood by either increasing the amount of HbF in their F-cells and/or by increasing the proportion of F-cells. The response to HU varies from patient to patient. If the magnitude of the HU-elicited increase in the %HbF (with respect to the total Hb) of the patient's blood could be predicted, "non-responders" could be identified. This would provide useful data for clinicians, including the ability to differentiate non-responding patients from those that are non-compliant as well as the ability to predict whether a patient's sickle cell symptoms will be significantly reduced by HU therapy.

Materials and Methods

- **Eligibility Criteria and HU Dosage**

Sickle cell anemia ($\alpha_2\beta^s_2$) patients at least 16 years of age were offered HU if they had one or more of the following: a minimum of three vaso-occlusive crises per year; severe anemia/hyperhemolysis (Hb <6.5 g/dl and bilirubin >4.0 mg/dl); fatigue; history of acute chest syndrome; or leg ulcers. The risks and benefits of HU therapy were discussed with each patient, and the patients were given written information describing HU and its effects. The potential teratogenic effects of the drug were emphasized. Patients undergoing treatment with HU were advised against pregnancy or fathering a child.

The sickle cell patients were treated with a daily, oral dose of HU (15-28 mg/Kg of body weight), with the dose increasing over time. The dose was increased when HbF levels stabilized in two consecutive monthly visits. If there was evidence of bone marrow suppression, the HU was withheld and, when the marrow had recovered (~one week), HU treatment was resumed at a lower dose. Only patients who had received HU for 8 months or longer were included in the ANN studies, although some patients first became positive responders well after 8 months of continuous treatment. The data from eighty three patients met this criterion.

- **Collection and Analysis of Blood Samples**

Blood samples were obtained and analyzed monthly. The values of 23 parameters (see Table 1) for each of the 83 patients were recorded for a minimum of 8 months and a maximum of 98 months. The parameters analyzed for each patient are those normally included in a 'Complete Blood Count' (CBC), 'Chemistry Profile' (SMA-18)' serial HbF levels (absolute and percent), DNA analyses for β globin gene haplotype and number of α genes, treatment duration, weight, age, and gender. The values of these parameters were used to train ANNs and for statistical analyses

- **Statistical Analyses**

One-tailed student T-test (a function of Microsoft Excel 7. 0) was applied to the results of statistical analyses to confirm that the observed variation in concentration of HbF as well as other results were due to the HU administered and not due to random events. A confidence level of 0.001 was the minimum value accepted as significant in the results of Student T-tests. Patients lacking values for critical parameters were eliminated from this study. For example, in studies of the correlation between final HbF concentration and mean cell volume, all patients were excluded for whom the 'mean cell volume' or the 'final HbF concentration' was missing.

Table 1. A description of the 23 parameters for which data was obtained from the patients.

| Parameter | Description | Units |
|-----------|--|--------------------------|
| Age | Age of patient at the time of analysis | Days |
| Sex | Male/Female | F=1, M=2 |
| NAGG | α Globin gene number | None |
| BAN | Number of BAN haplotypes | 1,2, or 3* None |
| BEN | Number of BEN haplotypes | 1,2, or 3* None |
| CAM | Number of CAM haplotypes | 1,2, or 3* None |
| SEN | Number of SEN haplotypes | 1,2, or 3* None |
| WGT | Weight of patient | kg |
| %HbF | Fetal hemoglobin, as % total hemoglobin | None |
| HbF | Fetal hemoglobin, absolute value | g/L of blood |
| Hb | Total hemoglobin concentration | g/dL of blood |
| RBC | Red blood cell count | $\times 10^{12}$ / Liter |
| PCV | Packed cell volume (hematocrit) | Liter / Liter |
| RDW | % Variation in the size of red cells | None |
| Retic | Reticulocytes | $\times 10^3$ |
| MCV | Mean cell (erythrocyte) volume | Femtoliters |
| MCH | Mean cell hemoglobin | Picograms |
| WBC | White cell count | $\times 10^9$ / Liter |
| Polys | Polymorphonuclear leukocytes | $\times 10^9$ / Liter |
| Plats | Platelet count | $\times 10^9$ / Liter |
| Bili | Bilirubin concentration in blood | mg / dL |
| NRBC | Nucleated red blood cells seen in peripheral blood | number per WBC |
| Duration | Duration of treatment a patient received to arrive at the maximum %HbF level | Days |

*The actual values were 0,1,or 2, but zero could not be used (see last paragraph under ANN Analyses).

- **ANN Analyses**

Several options are selected for the design and operation of each ANN. The two neural network models used for the experiments reported in this manuscript are one- and two-stage, fully connected, feed-forward topology with a back-propagation (Delta rule, in the case of the single stage network) learning algorithm. We used a step size of 0.01 with no momentum term³⁸. The first of the two different neural network architectures used for this project consisted of 23 input neurons and one output neuron (no hidden neurons) (23-1) and the second network consisted of 23 inputs, 4 hidden neurons and one output neuron (23-4-1). The first network architecture was used to predict whether treatment with HU results in a doubling of the HbF concentration in the patient's blood. The second network architecture was used to predict whether

treatment with HU would raise the HbF concentration in the blood above a predetermined threshold.

Three neural network simulation programs were used in the experiments described in this paper. The first software package was developed in house and is copyright protected. If desired, a copy of the executable version of the software for ULTRIX, DIGITAL UNIX, and SGI's IRIX 6.4 can be acquired by contacting the authors. The two other software packages are NeuroShell 2.0 and Partek 2.0 (by Ward systems group, Inc. [Fredrick, MD] and Partek Inc.[St. Charles, MO], respectively). Multiple packages were used to help ensure that we were not using irreproducible software-specific features. The results of each of the three software packages are comparable.

NeuroShell 2.0 and Partek 2.0 were used to train ANNs and to select those parameters that had an observable impact (when omitted from the training) on the ability of the ANNs to predict a patient's HbF response to HU. NeuroShell 2.0 selected the "most influential parameters" by monitoring, during training of ANNs, the relative activity and strength (weight) of the connections between the neurons; those parameters that had active neurons and strong connections are considered important contributors to the training. All NeuroShell experiments were repeated five times to eliminate the effects of random initialization. Partek identified the parameters with greatest impact by training a series of ANNs, each time removing a different one of the 23 parameters from the training set. The influential parameters were identified by the effect that removing the parameter had on the ability of the ANN to correctly predict the response to HU, that is, how much did the predictive ability of the trained ANN deteriorate in the absence of the parameter; the greater the degradation in the ability of the ANN, the more influential the parameter. Partek experiments were repeated with two different seeds for the random number generator which we used to create the initial condition of the ANNs. The experiment was repeated five times for each seed to ensure reproducibility. The average of these ten runs constituted the results of Partek software. Only the results of the NeuroShell software are listed in this report since both software produced similar results.

Some patients did not have data for all 23 parameters. Since the presence of a zero at the input during the training causes the backpropagation algorithm not to update the first stage weights connected to that input, we decided to substitute a zero for each missing parameter. In other words, the weight updating (learning) of the first stage is done only based on the value of the nonzero input parameters. This provided away of reducing the effects of the missing data on the overall learning results.

Patients with three or more missing parameters were excluded from the analyses. Since ANNs associate a special meaning (wild card) with the number zero, all of the parameters which may have a valid measurement of zero were shifted by one (add 1 to the value). For example the number of SEN haplotypes may be zero. In such cases the number of haplotypes presented to the ANN are all shifted (increased) by one. The shifting principle is applied to all four β globin haplotypes (Bantu, Benin, Cameroon, Senegal) and to nucleated red blood cell counts (NRBC). The ANN algorithm looks for differences between input values; therefore, the differences between 1, 2, and 3 are equivalent to the differences between 0,1,and 2. These

procedures, in conjunction with other considerations (e.g. duration of treatment), resulted in the database of 83 patients.

Results

- **Statistical Analysis**

Some interdependent biological phenomena interact in a nonlinear manner, while other interactions exhibit a linear relationship. The existence and strength of linear relationships can be determined by correlation coefficient analysis, a statistical method^{40, 41}. The results of correlation coefficient analysis fall between +1 and -1, where values close to +1 or -1 are indicative of a strong linear relationship between the phenomena being examined, and values close to 0 indicate a weak or non-existent linear relationship.

Correlation coefficient analysis was used to determine whether, in sickle-cell patients administered HU, a linear relationship exists between the magnitude of the increase in HbF concentration and the change in the value of each of the other parameters taken independently. The results of these analyses (Table 2) revealed that there are no significant linear relationships between the HU-induced increase in HbF concentration and any of the other parameters. Although the HbF concentration appears to correlate most strongly with the length of time patients are treated with HU, even the value of this correlation coefficient, 0.45, is too small to be significant; a significant correlation in this study has an absolute value of 0.8 or higher. Furthermore, there is not even a linear relationship between the HbF concentration of patients prior to HU treatment and the HbF concentration following HU treatment (Figure 1). Perusal of the data presented in Figure 1 reveals that the magnitude of the increase in HbF concentration in response to HU therapy is unrelated to the concentration of HbF in the patient's blood prior to HU therapy. Thus starting with a low concentration of HbF is not a disadvantage in reaching an HbF concentration of 15% of total Hb, and starting with a high concentration of HbF is likewise no advantage in this regard.

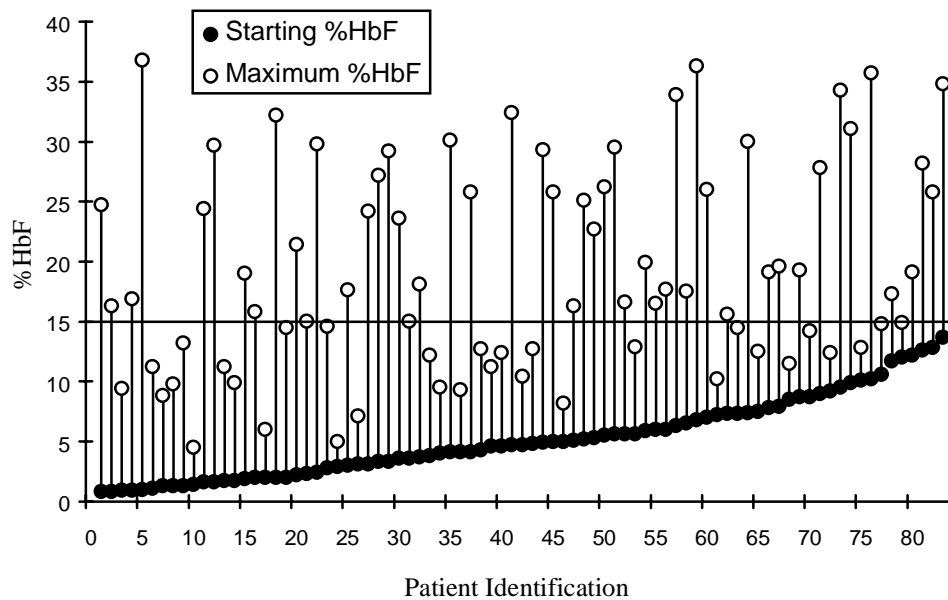


Figure 1. The % of total Hb accounted for by HbF in the blood of 83 sickle cell patients before HU treatment (filled circles) is unrelated to the %HbF after HU treatment (open circles) . Those patients whose HbF concentration did not exceed 15% prior to HU treatment and that did exceed 15% HbF after HU treatment are considered responsive.

The absence of linear relationships between the HbF concentration and one or more of the parameters examined (Table 2) suggests that either the values of the parameters are not related to the effect of HU treatment on HbF concentration or that the parameters are related in a nonlinear manner (e.g., exponential, or higher order polynomial³⁸). ANNs are particularly well suited to analyze complex, nonlinear relationships. This attribute of ANNs assisted us in the discovery, in sickle cell patients, of non-linear relationships between the magnitude of the HU-induced increase in HbF concentration and the values of other parameters (Table 1).

Table 2. Correlation coefficients between the increase in HbF concentration following HU treatment and the effect of HU on the value of the indicated parameter (sorted in descending order).

| Parameter Name | Correlation Coefficient | Minimum | Maximum |
|----------------|-------------------------|---------|---------|
| Duration | 0.45 | 27 | 3763 |
| Age | 0.35 | 6332 | 19461 |
| RBC | -0.23 | 1.45 | 4.58 |
| RDW | -0.21 | 15.7 | 34.5 |
| PCV | -0.19 | 0.132 | 0.351 |
| HbF | -0.18 | 0.5 | 13.4 |
| Hb | -0.18 | 4.6 | 11.5 |
| WBC | 0.18 | 4.6 | 25.9 |
| Sex | -0.16 | - | - |
| %HbF | -0.15 | 0.8 | 13.7 |
| CAM | 0.15 | 0 | 1 |
| MCH | 0.14 | 21.9 | 44.9 |
| Polys | 0.14 | 0.89 | 20.7 |
| Plats | -0.13 | 118 | 1414 |
| MCV | 0.13 | 72.7 | 126.8 |
| BAN | -0.12 | 0 | 2 |
| BEN | 0.12 | 0 | 2 |
| Retic | 0.10 | 48.6 | 876.8 |
| SEN | 0.07 | 0 | 2 |
| Bili | -0.07 | 0.6 | 15.1 |
| NBRC | 0.03 | 0 | 91 |
| WGT | -0.02 | 40.9 | 90.9 |
| NAGG | -0.01 | 2 | 4 |

- **Effect of HU on the HbF concentration in the blood**

Examination of the concentrations of HbF and HbS and the volume of the red blood cells of sickle cell patients provide a quick measure of how the patients are responding to HU therapy. The mean values of these three parameters obtained from 83 patients before and after treatment with HU are presented in Table 3. The HU-induced increase in HbF concentration and in the average volume of the red blood cells were shown to be significant at the 0.001 level by a Student T-test. The same test indicated that the small difference in HbS concentration before and after HU treatment is not significant. The increases in HbF concentration and mean volume of red blood cells are in agreement with results of previous reports^{42,43,44,45}.

Table 3. Mean values. before and after treatment with HU, for three parameters of 83 sickle-cell patients.

| Average of 83 patients | Before HU | After HU |
|----------------------------|-----------|----------|
| HbF Conc. (g/L) | 4.4 | 19.1 |
| HbS Conc. (g/dL) | 7.7 | 7.4 |
| Red Blood Cell Volume (fl) | 92.5 | 112.9 |

The concentration of HbF in the blood of each of the 83 sickle-cell patients, before and during treatment with HU, is plotted in Figure 2. The significance of the difference in HbF concentration before and after HU treatment is made even more apparent by the lack of an effect of HU treatment on the HbS concentration (compare the data in Figure 2 with that in Figure 3). HU treatment increased the mean HbF concentration of the 83 patients from 4.4 to 19.1 grams per liter (Table 3). The one-directional spread in the concentration of HbF following treatment with HU is evidence that the individual members of the patient population do not respond equally to HU. Furthermore, since there are no reports of HU causing a decrease in the HbF concentration of blood, it is reasonable to conclude (by observing the data in Figure 2) that some patients with low initial HbF levels (Figure 2) are weakly effected by HU and thus remain at the low end of the distribution. This conclusion is supported by the data in Figure 1.

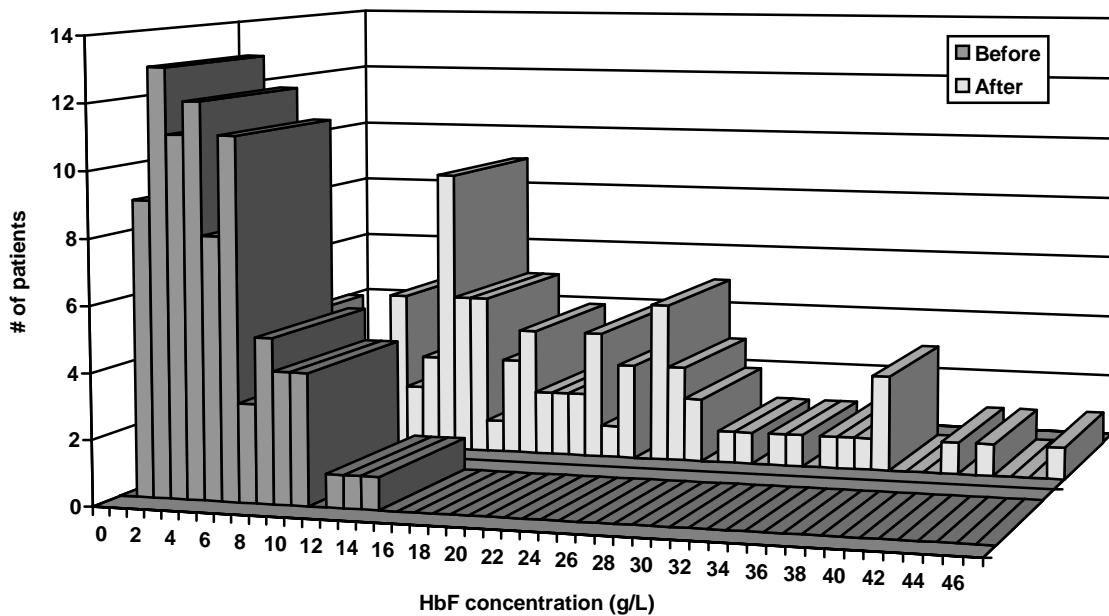


Figure 2. The distribution of the initial and maximum HbF concentrations of the 83 sickle cell patients prior to and after treatment with HU.

- **Effect of HU on the concentration of HbS in red blood cells**

HU increases the HbF concentration in the blood of sickle cell patients by increasing the number of F-cells and/or the amount of HbF of individual F-cells. However, at the beginning of this study it was unclear whether HU also alters the HbS concentration of the blood. An increase in the HbS concentration of blood would likely be detrimental since it is this relatively insoluble form of hemoglobin that, in conditions of low oxygen concentrations, aggregates causing the sickling of the erythrocytes.

The HbS concentration in the blood of the 83 patients, before and after HU treatment, is summarized in Figure 3. The results establish that the HbS concentration in the blood is not significantly affected by HU treatment. In summary, HU raises the HbF concentration of blood whereas HU has no apparent effect on the HbS concentration.

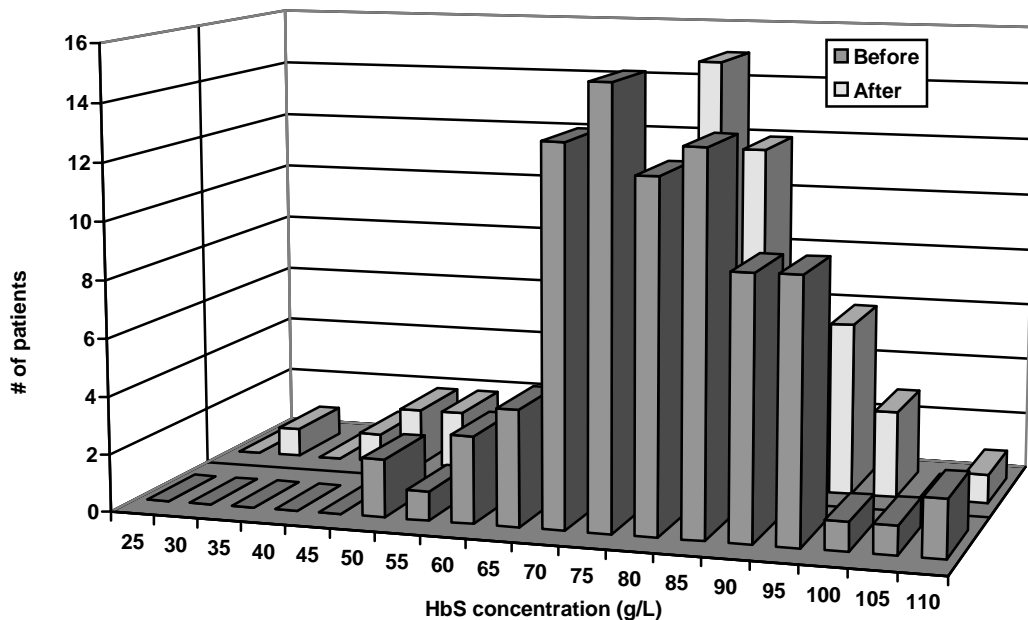


Figure 3. The distribution of the initial and final concentration of HbS in the blood of 83 sickle cell patients treated with HU.

- **Effect of HU treatment on the average volume of the red blood cells of sickle cell patients**

The mean cell volume (MCV) of erythrocytes of most but not all patients increases significantly following treatment by HU (shown by a Student T-test to be a significant increase). The mean cell volume of the erythrocytes of each patient, both before and after at least eight months of HU therapy, is compared in Figure 4. The average HU-induced increase in the volume of the red blood cells of the 83 patients is 22%. This agrees with the results of an earlier report^{42,43,44,45}. Thus monitoring the MCV may be a useful guide for determining compliance with HU treatment.

It is interesting to compare the HU-induced increase in HbF concentration with the increase in MCV. If the relationship between the change of these two parameters were linear, then the correlation coefficient would indicate the strength of this relationship. However, since the increase in MCV is not linearly dependent on the increase in HbF concentration (see above), the correlation coefficient is only a rough linear estimate of the strength of the relationship. The data appear to approximate a logarithmic relationship (Figure 5).

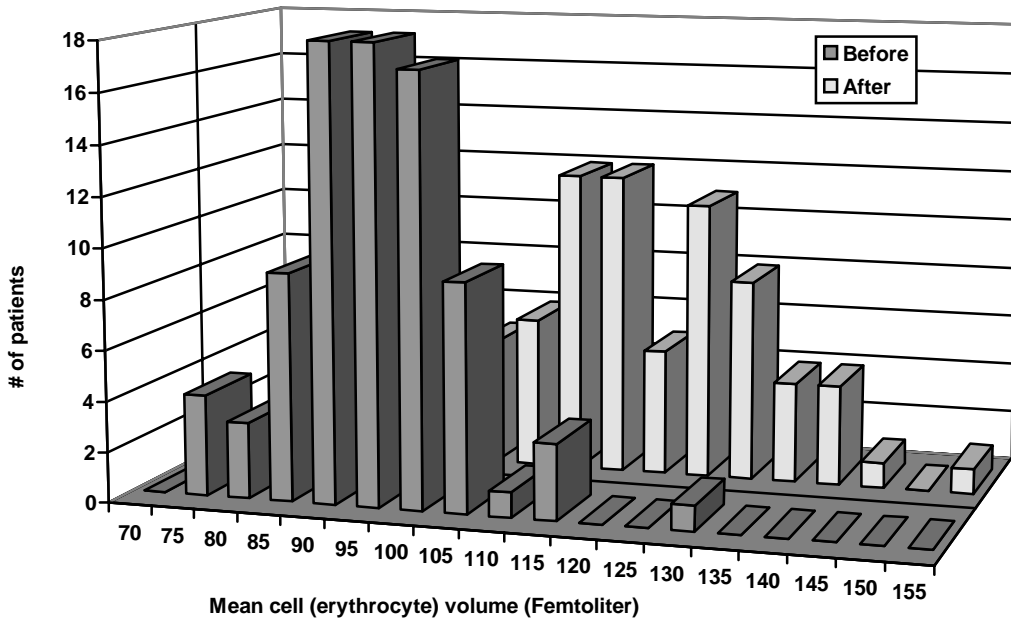


Figure 4. The distribution of average volume of the red blood cells of 83 sickle cell patients before and after treatment with HU.

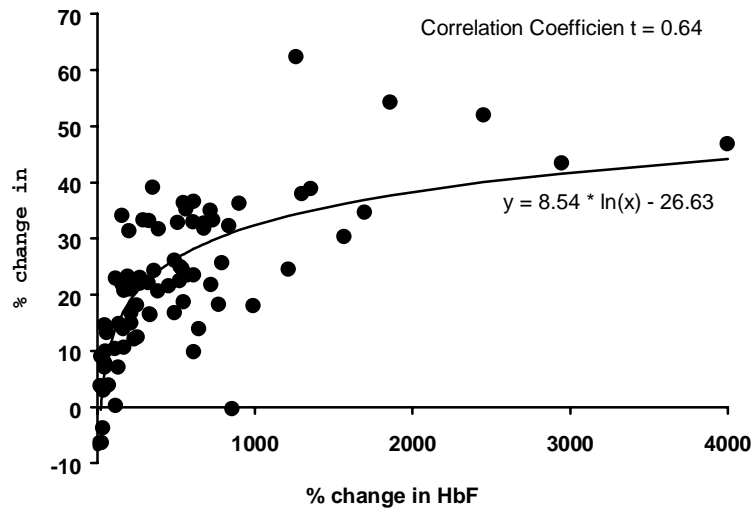


Figure 5. % change in HbF concentration versus % change in MCV measured before and after treatment with HU.

- **Training and testing of ANNs to predict the effect of HU treatment on the HbF concentration of the blood of sickle cell patients**

The data from the 83 participating patients needed to be separated, before training ANNs, into a group that responds to HU and a group that does not respond. The criterion for responders was first defined as a doubling of the patient's initial HbF level. Thus a patient whose blood HbF concentration changed in response to treatment with HU by increasing, for example, from an initial value of 0.5 g/L to a final value of 1.0 g/L would be considered a "responder," while a patient whose blood concentration of HbF increased from 20 to 35 g/L would be considered a non-responder. Since the clinical correlation of a doubling in HbF is an ambiguous criterion, we decided to distinguish responders from non-responders based on the HbF concentration passing a threshold of 15% of total Hb. The selection of the threshold is based on published reports that HbF has a beneficial effect at this concentration^{43,44,46}. This threshold divides the 83 patients into 58% responders and 42% non-responders (Figure 6).

An ANN using 23 input neurons, 4 hidden neurons, and one output neuron was used for the 15% threshold experiment. This neural network produced an output value higher than 0.5 if the patient was predicted to be a responder and an output of less than 0.5 if the patient was predicted to be a non-responder. The individuals whose initial HbF concentration exceeded the 15% threshold were excluded from that particular experiment in order to maintain the coherence of the threshold criterion. Three patients were eliminated from these experiments based on this criterion, which resulted in 83 patients qualifying for this experiment.

The threshold experiment was designed in such a way as to eliminate the possibility that the ANN could simply "memorize" the values of the parameters of each

patient. This was accomplished by training ANNs with the parameter values of 82 of the patients, and then using the values of the patient whose parameters had not been seen by the ANN to test the ANN. This procedure was repeated 83 times and each time an ANN was trained (a different patient was left out of the training). The result of this experiment is presented in Figure 6. Seventy patients were correctly classified as responders or non-responders while 13 were misclassified. Thus 84% of the responses were predicted correctly. This experiment was repeated five times with, on average, 86.6 correct predictions with a standard deviation of +/-2.0.

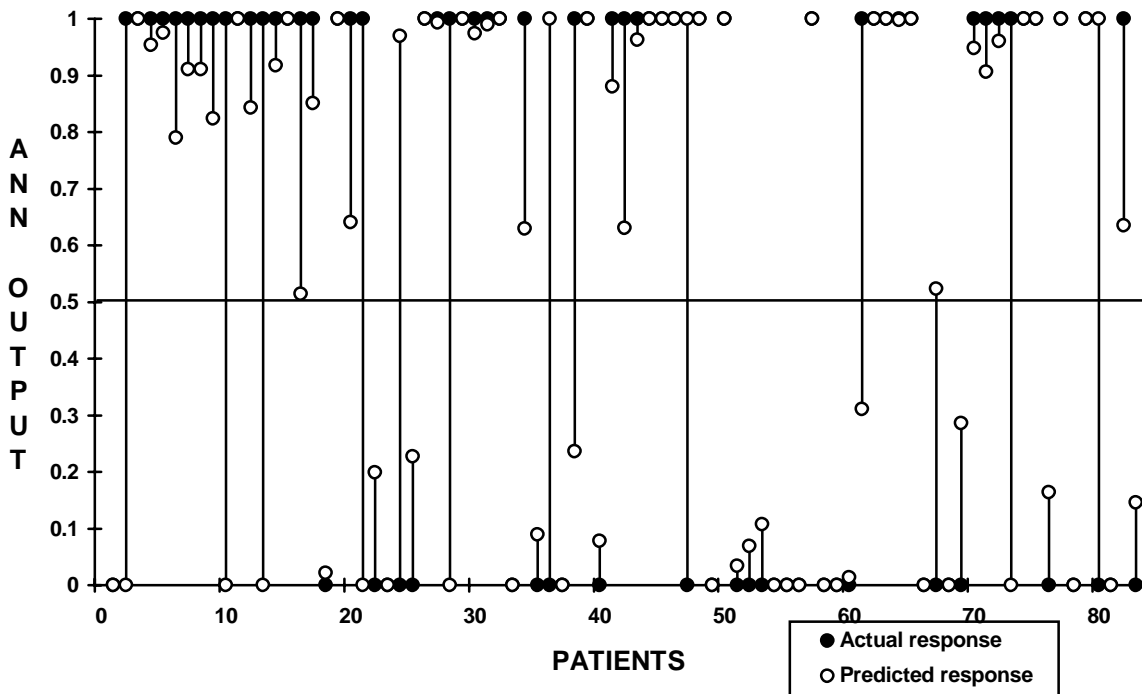


Figure 6. The prediction by ANNs of which patients would respond to HU by an increase in their HbF concentration to the point where it accounts for 15% or more of their total Hb. ANNs were trained with the values of the parameters of 82 patients and then tested with the values of the parameters of the patient that had not been used to train the ANN. This procedure was repeated 83 times, each time leaving out a different patient and training the ANN with the data from the other 82 patients to give the values in the figure. Patients whose HbF concentration did not reach 15% of the total Hb should have generated an ANN “output” of less than 0.5, while patients whose HbF concentration exceeded 15% of the total Hb should have generated an ANN output of more than 0.5.

- Contribution of each of the 23 parameters toward enabling ANNs to predict which sickle cell patients will respond to HU treatment by an increase of their HbF concentration to greater than 15% of total Hb.

This experiment was designed to identify which of the 23 parameters are most important or influential in assisting ANNs to predict those patients that will respond to

HU treatment. Response, in this section is defined as the increase in HbF concentration to more than 15% of total Hb. Determining the importance of each of the 23 parameters was accomplished by employing two different methods. The first method consisted of a recursive elimination process in which a different set of parameters was taken out of the training set. The ANNs were trained with the values of the remaining parameters. The software measures the degradation of performance due to the missing parameters. This experiment is an exhaustive elimination process in which the removal of every combination of parameters ($2^{23}-1=8,388,607$ combinations) is evaluated. The degradation (or importance) of the parameters observed are the averages of 10 experiments (2 different seeds, and 5 runs per seed) performed on Partek software. The final effect of removing each set of parameters is calculated by averaging the performance degradation of the 10 ANNs trained without that set of parameters.

The second method of parameters selection was implemented on the NeuroShell software. This software performs a different method of parameter selection. This algorithm is initiated by setting equal values for each parameter. During the course of training, these values are updated to reflect the strength of the synaptic connection associated with the particular parameter as well as its contribution towards the discovery of the correct answer. Thus at the end of the training the contribution of each parameter reveals its importance in the solution of the problem. Each training session was repeated 5 times to eliminate any random behavior of the system.

Although the above two methods are two distinctly different ways of parameter selection, both algorithms produced similar in extracting the valuable parameters. Therefore, only the results of the NeuroShell parameter selection are shown in this report.

The 23 parameters and their scores, which are proportional to their contributions in predicting the response to HU treatment, are listed in Table 4. This table contains the averaged data for over 5 different training sessions. The lack of any particularly influential contributors indicates that no one parameter contains most of the information needed to predict the response to HU. Therefore, based on the given contributions, it is reasonable to assume that the information needed for a successful classification is distributed among a number of parameters, perhaps even a fairly large number of parameters.

The ANNs trained in Figure 6 used the values of all 23 parameters. A separate experiment was carried out to determine if the values of just ten 10 of the 23 parameters listed in the previous section could be used while maintaining the ANN's full ability to identify responders and non-responders. This experiment used the top 10 parameters listed in Table 4. The ability to eliminate unnecessary parameters has the potential for reducing the problem size by more than 50% and might assist in elucidating the mechanisms by which ANNs function.

Table 4. The effectiveness of each of the 23 parameters to assist ANNs in predicting the response of patients to HU treatment.

| Parameter | Score |
|------------------|--------------|
| Duration | 0.083 |
| RDW | 0.063 |
| WBC | 0.059 |
| Plats | 0.053 |
| MCV | 0.053 |
| Polys | 0.052 |
| WGT | 0.050 |
| SSEN | 0.045 |
| Retic | 0.043 |
| Sex | 0.042 |
| SCAM | 0.041 |
| NAGG | 0.041 |
| Hb | 0.040 |
| SBAN | 0.040 |
| MCH | 0.035 |
| RBC | 0.034 |
| SBEN | 0.034 |
| Bili | 0.034 |
| Age | 0.033 |
| HbF | 0.032 |
| %HbF | 0.031 |
| PCV | 0.031 |
| SNBRC | 0.030 |

Discussion

Artificial neural networks capable of recognizing patterns can do so by memorization or generalization^{38,40}. Memorization occurs when the ANN can memorize all or some of the training patterns exactly as they are. Generalization occurs when the ANN is not large enough to memorize the training pattern(s) exactly, but rather learns the identifying feature(s) of each pattern. In cases such as predicting a drug response, memorization is not desired as it will cause the network to perform well in predicting the response of the patients included in the training set but will fail when tested with patients not included in the training set (new patients). The type of ANN used for the experiments described in this manuscript is, in theory, capable of memorizing the values of the 23 parameters for each of the 83 patients. We were concerned that we could be misled into thinking we were observing a feature detecting pattern recognition procedure when in fact the computer had memorized the 83 sets of data. We avoided the possibility of being misled in this way by training an ANN with the parameter values of 82 patients. The parameter values were obtained before the patients were exposed to hydroxyurea, and then the trained ANN was tested with the parameter values, also obtained before exposure to hydroxyurea, of a single patient that had been excluded from the training set so that the trained ANN had never seen the patient's parameter values and, therefore, could not have memorized them. We repeated the training of 83 different ANNs, that is, each time we started with a different ANN that had been "initiated" by assigning random weights to the connections between its neurons, and each time we omitted the parameter values of a different patient and used the parameter values of that patient to test the system. This experiment was repeated in its entirety five times, that is, each repeat required training 83 ANNs. The system was asked, for each of the 83 patients, whether treatment with hydroxyurea would increase the HbF concentration to greater than 15% of the blood's total Hb. The system, under these conditions, correctly predicts the response of $86.6 \pm 2.2\%$ of the patients.

Some misclassified patients in all likelihood had values that fell on the edge of the envelope formed by the values of the 82 individuals in the training sets. We conclude this because, when the experiment was repeated five times, some of the 13 patients miss-classified in the first experiment were correctly assigned in one or more of the repeat experiments and vice versa. Since the starting weights of the connections between the neurons of the 83 training sets are randomized before each training session, variations in the classification of a small percentage of the patients is expected.

There are several factors that can be corrected or improved to increase the accuracy of the ANNs. A larger data set (training set) would ensure a more robust predictor. A more accurate and complete data set would do likewise. ANNs are powerful pattern recognition engines if the data by which they are trained is robust. We expect the accuracy of the predictions made by ANNs vis-à-vis the response to HU to improve if the ANNs can be trained with the parameter values obtained from a larger

number of appropriately treated sickle-cell patients and if the ANN architecture and algorithm are optimized.

We have demonstrated that it is possible to identify (with ~85% accuracy) patients who will respond to HU therapy by an increase in their HbF such that it equals 15% or more of the patient's total Hb. . A separate study might refine the level of HbF needed to result in a significant improvement in the clinical course of the disease. It is possible that sickle-cell patients at different points in the progression of the disease and at different ages may require different levels of HbF for meaningful relief.

Training ANNs to distinguish responders from non-responders is of value regardless of how the two classes are defined by the medical community. An important contribution of the research reported herein is that ANNs can be used to detect correlations between medical data that humans, without the assistance of computers, can not see. There are many areas in medicine where data analysis is likely to be substantially improved by utilizing the power of pattern recognition software. A related contribution of this research, and perhaps the most important for future studies is that important, unforeseen information encrypted in the results of standard blood analyses can be deciphered by ANN-assisted analyses.

References

1. T. H. Papayannopoulou, T. C. Mcguire, G. Lim, E. Garzel, P. E. Nute & G. Stamatoyannopoulos. Identification of Haemoglobin S in red cells and normoblasts, using fluorescent anti-Hb S antibodies. *Br J Haematol* 34: 25-31 (1976)
2. D. Chiu, E. Vichinsky, M. Yee, K. Kleman & B. Lubi. Peroxidation, vitamin E, and sickle-cell anemia. *Ann N Y Acad Sci* 393: 323-35 (1982)
3. R. Hoover, R. Rubin, G. Wise & R. Warren. Adhesion of normal and sickle erythrocytes to endothelial monolayer cultures. *Blood* 54: 872-6 (1979)
4. S. C. Liu, L. H. Derick & J. Palek. Dependence of the permanent deformation of red blood cell membranes on spectrin dimer-tetramer equilibrium: implication for permanent membrane deformation of irreversibly sickled cells. *Blood* 81: 522-8 (1993)
5. J. L. Stephan, E. Merpit-Gonon, O. Richard, C. Raynaud-Ravni & F. Freycon. Fulminant liver failure in a 12-year-old girl with sickle cell anaemia: favourable outcome after exchange transfusions. *Eur J Pediatr* 154: 469-471 (1995)
6. H. J. Wolters, H. ten Cate, L. L. Thomas, D. P. Brandjes, A. van der Ende, Y. van der Heiden & L. W. Stadius van Eps. Low-intensity oral anticoagulation in sickle-cell disease reverses the prethrombotic state: promises for treatment? *Br J Haematol* 90: 715-717 (1995)
7. T. L. Yarboro. The natural history of homozygous S sickle cell anemia in two sisters. *J Natl Med Assoc* 87: 363-368 (1995)
8. F. Lori, A. Malykh, A. Cara, D. Sun, J. N. Weinstein, J. Lisziewicz & R. C. Gallo. Hydroxyurea as an inhibitor of human immunodeficiency virus-type 1 replication. *Science* 266: 801-805 (1994)
9. Griffin P. Rodgeres, M.D., George J. Dover, M.D., Constance Tom Noguchi, Ph.D., Alan N. Schechter, M.D., and Arthur W. Nienhuis, M.D. Hematologic responses of patients with sickle cell disease to treatment with hydroxyurea. *The New England Journal of Medicine*. April 1990, vol 322, No.15 p 1037-1044
10. R. N. Haire, W. A. Tisel, G. Niazi, A. Rosenberg, S. J. Gill & B. Richey. Hemoglobin solubility as a function of fractional oxygen saturation for hemoglobins in polyethylene glycol: a sickle hemoglobin model. *Biochem Biophys Res Commun* 101: 177-82 (1981)
11. A. R. Hargens, L. J. Bowie, D. Lent, S. Carreathers, R. M. Petters, Hammel & P.F. Scholander. Sickle-cell hemoglobin: fall in osmotic pressure upon deoxygenation. *Proc Natl Acad Sci U S A* 77: 4310-2 (1980)
12. Adams RJ, Kutlar A, McKie V, Carl E, Nichols FT, Liu JC, McKie K, Clary A. Alpha thalassemia and stroke risk in sickle cell anemia. *Am J Hematol* 45 (4): 279-282 (1994)

13. Valafar, F., H. Valafar, O. K. Ersoy, and R. J. Schwartz. 1995. Comparative studies of two neural network architectures for modeling of human speech production. *Proceedings of the International Conference on Neural Networks (IEEE-ICNN '95)*, Perth, Australia 4: 2056-2059 (November 27-December 1).
14. Valafar, F. and O. K. Ersoy. 1996. PNS modules for the synthesis of parallel self-organizing hierarchical neural networks. *International Journal of Circuits, Systems, and Signal Processing* 15(1): 23-50.
15. Akay, M. 1993. A unified framework for using neural networks to build QSARs. *J. Med. Chem.* 36: 3565-3571.
16. Akay, M., Y.M. Akay, and W. Welkowitz. 1992. Neural networks for the diagnosis of coronary artery disease. *Proceedings of the International Joint Conference on Neural Networks*, Baltimore, MD (June).
17. Akay, M. 1992. Noninvasive diagnosis of coronary artery disease using a neural network algorithm. *Biological Cybernetics* 67: 361-367.
18. Alpsan, D. 1994. Auditory evoked potential classification by unsupervised ART 2-A and supervised fuzzy ARTMAP networks. *International Conference on Neural Networks (ICNN '94)*, IEEE, Orlando, FL (June 26-July 2).
19. Andrea, T.A. and H. Kalayeh. 1991. Applications of neural networks: quantitative structure-activity relationships of dihydrofolate reductase inhibitors. *J. Med. Chem.* 34: 2824-2836.
20. Andreassen, H., H. Bohr, J. Bohr, S. Brunak, T. Bugge, R.M.J. Cotterill, C. Jacobsen, P. Kusk, and B. Lautrap. 1990. Analysis of secondary structure of the human immunodeficiency virus proteins by computer modelling based on neural network methods. *J. Acquired Immune Deficiency Syndrome* 3: 615.
21. Apolloni, B., G. Avanzini, N. Cesa-Bianchi, and G. Ronchini. 1990. Diagnosis of epilepsy via backpropagation. *Proceedings of the 1990 International Joint Conference on Neural Networks*, Washington, DC 2: 571-574.
22. Armentrout, S.L., J.A. Reggia, and M. Weinrich. 1994. A neural model of cortical map reorganization following a focal lesion. *Artificial Intelligence in Medicine* 6(5): October.
23. Armstrong, W.W., R.B. Stein, A. Kostov, M. Thomas, P. Baudin, P. Gervais, and D. Popovic. 1993. Application of adaptive logic networks and dynamics to study and control of human movement. *Proceedings of the Second International Symposium on 3D Analysis of Human Movement*, Poitiers, France, pp. 81-84 (June 30-July 3).
24. Armstrong, W.W., A. Kostov, R.B. Stein, and M.M. Thomas. 1995. Adaptive logic networks in rehabilitation of persons with incomplete spinal cord injury. *Workshop on Environmental and Energy Applications of Neural Networks*, Richland, WA, Pacific Northwest National Laboratory (March 30-31).
25. Asada, N. and K. Doi. 1990. Potential usefulness of an artificial neural network for differential diagnosis of interstitial lung disease: pilot study. *Radiology* 177: 857.

26. Asada, N., K. Doi, H. MacMahon, S. Montner, M.L. Giger, C. Abe, and Y.Z. Wu. 1990. Neural network approach for differential diagnosis of interstitial lung diseases. *Proceedings of the SPIE (Medical Imaging IV)* 1233: 45-50.
27. Ashenayi, K., Y. Hu, R. Veltri, R. Hurst, and R. Bonner. 1994. Neural network based cancer cell classification. *Proceedings of the World congress on Neural Networks*, San Diego, CA I: 416-421 (June 5-9).
28. Astion, M.L. and P. Wilding. 1992. The application of backpropagation neural networks to problems in pathology and laboratory medicine. *Arch. Pathol. Lab. Med.* 116: 995-1001.
29. Astion, M.L. and P. Wilding. 1992. Application of neural networks to the interpretation of laboratory data in cancer diagnosis. *Clin. Chem. (US)* 38: 34-38.
30. Avanzolini, G., P. Barbini, and G. Gnudi. 1990. Unsupervised learning and discriminant analysis applied to identification of high risk postoperative cardiac patients. *Int. J. Bio-Med. Comput.* 25: 207-221.
31. Barski, L.L., R.S. Gaborski, and P.G. Anderson. 1993. A neural network approach to the histogram segmentation of digital radiographic images. In: *Intelligent Engineering Systems Through Artificial Neural Networks* (Dagli, Burke, Fernandez, and Ghosh, Eds.) 3: 375-380. *ASME Press*, New York, NY.
32. Bartels, P.H., D. Thompson, and J.E. Weber. 1993. Diagnostic decision support by inference networks. *In Vivo* 7: 379-385.
33. Baxt, W.G. 1990. Use of an artificial neural network for data analysis in clinical decision-making: the diagnosis of acute coronary occlusion. *Neural Computation* 2: 480-489.
34. Baxt, W.G. 1991. Use of an artificial neural network for the diagnosis of myocardial infarction. *Annals of Internal Medicine* 115: 843-848.
35. Echauz, J. and G. Vachtsevanos. 1994. Neural network detection of antiepileptic drugs from a single EEG trace. *Proceedings of the IEEE Electro/94 International Conference*, pp. 346-351, Boston, MA (May 10-12).
36. Shadmehr, R. and D.Z. D'Argenio. 1990. A neural network for nonlinear Bayesian estimation in drug therapy. *Neural Computation* 2: 216-225.
37. Weinstein, J.W., K.W. Kohn, M.R. Grever, L.V. Rubinstein, A.P. Monks, D.A. Scudiero, L. Welch, A.D. Koutsoukos, A.J. Chiasusa, and K.D. Paull. 1992. Neural computing in cancer drug development: predicting mechanism of action. *Science* 258: 447-451.
38. Rumelhart, D.E. and J.L. McClelland. *Parallel Distributed Processing: Explorations in the Microstructure of Cognition*, Vol. 1 and 2. *MIT Press*, Cambridge, MA.
39. Heckerman, D. 1996. Learning with Bayesian Networks. *International Conference on Neural Networks (ICNN'96)*, Plenary, Panel and Special sessions, Plenary Speech PL8, pp. 250.

40. Valafar, F. 1994. Parallel Probabilistic Self-Organizing Hierarchical Neural Networks (PPSHNN). *Doctoral dissertation*, Electrical Engineering Department, Purdue University, West Lafayette, IN, Spring.
41. Moore D. S. and McCabe G. P., *Introduction to the Practice of Statistics*. ISBN 0-7167-1989-4
42. Charache S., Barton F. B., Moore R. D., et al, Hydroxyurea and Sickle Cell Anemia, Vol. 75 No. 6 *Medicine*, p300-325
43. Charache S., Terrin L. M., Moore R. D., et al, Effect of Hydroxyurea on the Frequency of Painful Crises in Sickle Cell Anemia, *New England Journal of Medicine* 332:1317-1322 (May 18), 1995.
44. Charache S, Dover G. J., Moore R. D., et al, Hydroxyurea: Effects on Hemoglobin F Production in Patients With Sickle Cell Anemia, , *Blood*, Vol 79, 10(May15), 1992: pp 2555-2565.
45. Steinberg M. H., Lu Z., Barton F. B., Terrin L. M., et al, Fetal Hemoglobin in Sickle Cell Anemia : Determinants of Response to Hydroxyurea, *Blood*, Vol89, No 3(February 1), 1997:pp 1078-1088
46. Powars D.R., et al, Is there a threshold level of fetal hemoglobin that ameliorates morbidity in sickle cell anemia? *Blood*, 1984 Apr; 63(4): 921-926.