Viscosity of mixtures of sickle and normal red cells at varying hematocrit levels

Implications for transfusion

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Viscosity (η) in a blood suspension is affected by the total hematocrit level (H_T) as well as by the deformability of the cells. The impact of these combined factors on the rheologic behavior of sickle cell suspensions and on guidelines for transfusion has not been explored fully. Therefore, the η of mixtures of washed normal (AA) and sickle (SS) red cells was determined in a rotational viscosimeter as a function of the hematocrit level of SS cells (H_S), H_T, oxygen tension (PO2), and shear rate. The ratio H_T:η can be taken as an index of potential oxygen delivery. The optimal H_T (for maximum H_T:η) became progressively higher as the H_S or the H_S:H_T ratio was lowered; at a given H_T, H_T:η rose with a decrease in H_S, especially at low H_S values. These data support the concept that simple transfusion alone is not as beneficial to the patient as exchange transfusion and that substantial benefit can be obtained by bringing the patient to very low H_S levels. The finding that η rose with H_T more steeply when the H_S:H_T ratio rather than H_S was held constant suggested that the absolute level of H_S may be more useful than the H_S:H_T ratio as a guide for a transfusion regimen. **TRANSFUSION** 1987;27:228-233.

PATIENTS with sickle cell anemia frequently receive transfusions to reverse or prevent some of the more severe complications of the disease, such as stroke or priapism. It is generally recognized, however, that transfusion can lead to an increase in blood viscosity, which may cause a worsening of existing infarctions or the initiation of new ones.[1,2] The safest method of transfusing a patient has not been established clearly. A ceiling of 35 percent for total hematocrit level (H_T) is generally accepted, but higher H_T values are occasionally achieved, whether deliberately or inadvertently.[3,4] The percentage of sickle hemoglobin (HbS) is limited by some transfusion programs to levels as low as 25 percent,[5] whereas others allow up to 50 percent.[2] Lessin et al.[6] and Anderson et al.[7] showed that blood viscosity increases with an increasing percentage of sickle cells (SS cells), but their analyses did not address the question of rising H_T. Jan et al.[1] showed that transfusion to an H_T above 35 percent caused an increase in blood viscosity sufficient to diminish the venous PO2 level, but they did not correlate their data with the relative percentage of SS cells. Our study was conducted to obtain a more complete profile of the rheologic behavior of mixtures of SS and normal (AA) red cells in different proportions and over a range of H_T, with the ultimate aim of determining the optimal conditions that can be attained by the transfusion of patients with sickle cell disease.

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Materials and Methods

Blood was taken from children who weighed more than 30 kg and were not chronically ill other than sickle cell disease. At least 6 weeks had elapsed from any previous crisis and 3 months from any transfusion. Informed consent was obtained from both the parent and the patient. The children were homozygous for the sickle gene; none had detectable hemoglobin A on electrophoresis, and A_2 levels were all less than 3.5 percent. The fetal hemoglobin level was less than 15 percent. Patients with mean corpuscular volume less than 80 μm^3 or greater than 100 μm^3 were excluded. AA blood was obtained from healthy adults.

The hematocrit level was determined by centrifugation at 15,000 $\times$ g for 5 minutes. The red cell counts were determined in a standard hemocytometer, and the mean corpuscular volume was taken from the ratio of hematocrit to red cells. Hemoglobin electrophoresis was performed on cellulose acetate at a pH value of 8.4 (Helena Laboratories, Beaumont, TX), and hemoglobin S was confirmed by metabisulfite sickle preparation. Fetal hemoglobin levels were measured by a modification of the alkali denaturation technique of Singer et al.[8] The hemoglobin A_2 level was assayed after separation on a commercially available column (Helena Laboratories).

The red cells in blood samples were washed three times and resuspended in a buffer solution containing 0.69 g per

dl NaCl, 0.227 g per dl NaHCO3, 0.037 g per dl KCl, and 0.5 g per dl bovine serum albumin, with pH brought to 7.4 by bubbling gas containing 20 percent O2 and 5.6 percent CO2 for approximately 30 minutes. Each experiment was performed at a given ratio of SS to AA cells, at two oxygen tensions, and over a range of H_T from 20 to 45 percent. The washed SS and AA cells were mixed to the desired ratio of the hematocrit level of SS cells (H_S) to H_T. The suspension was then divided into two aliquots for adjustment of H_T to 20 and 45 percent. The cells were not fully oxygenated when the hematocrit measurement was made; however, we have found that the hematocrit values of venous blood samples remained stable (within 0.3%) during progressive oxygenation. Each of the two aliquots was further divided into two parts, one to be brought to a PO2 value of more than 100 torr, and the other to 37 ± 1 torr. The adjustment of oxygen tension was performed in a rotary tonometer, using N2 containing 5.6 percent CO2 with or without 20 percent O2. The pH of the suspensions remained at 7.4. For each oxygenation state, the sample with $H_T = 20$ percent and the sample with $H_T = 45$ percent were mixed in various proportions without exposure to air to obtain samples with a range of H_T, all at the same PO2 value and with the desired SS:AA ratio. Measurements of viscosity of the cell suspensions were made in a coaxial cylinder viscosimeter at shear rates of 0.52 to 208 sec^{-1}. Light microscopic examination of the suspensions showed no cellular aggregation.

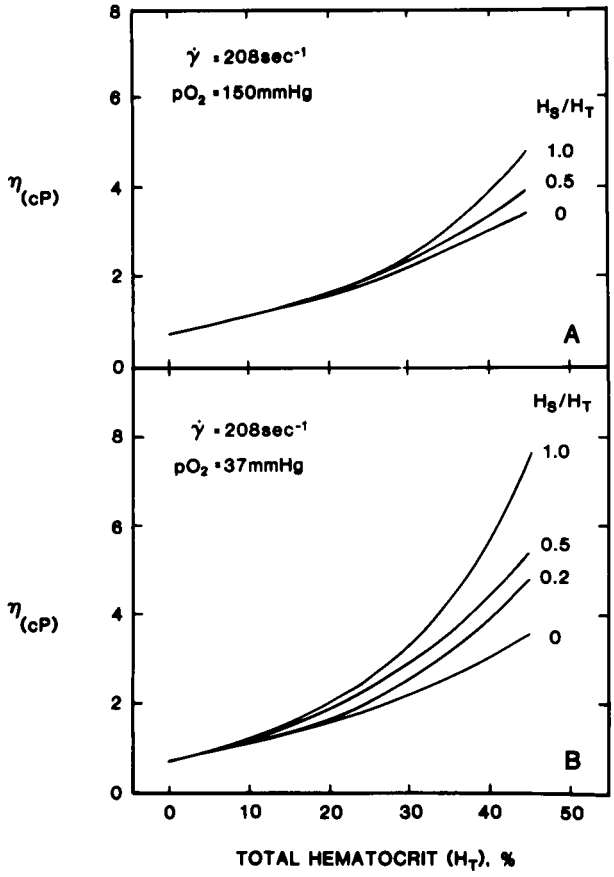

FIG. 1. The rise in viscosity (η) with H_T at given proportions of sickle cells to total cells in the suspension (H_S:H_T). *Top:* Oxygenated suspensions. *Bottom:* Deoxygenated suspensions.

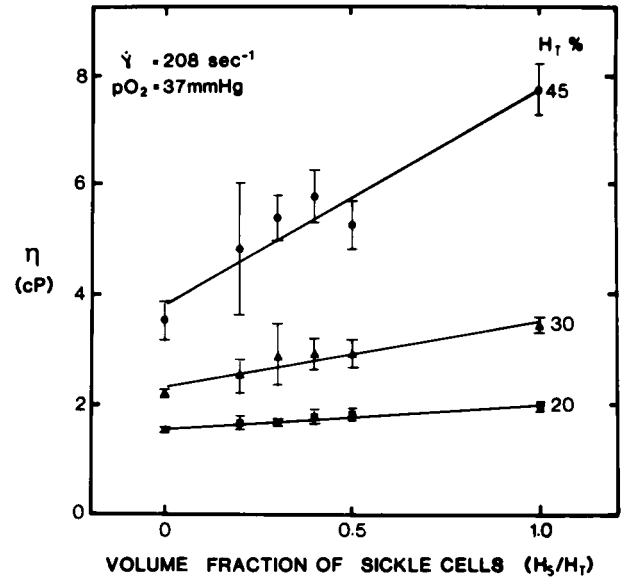

FIG. 2. A cross-plot of the data from Figure 1 (*bottom*), showing the linear rise in viscosity (η) with increasing H_S:H_T ratios at various H_T levels. At higher H_T values, the effect of rising H_S:H_T ratios on η is more marked.

Results

At a constant H_S:H_T ratio, the viscosity of oxygenated suspensions at a given shear rate ($\dot{\gamma}$) rose with H_T. The rise of viscosity with H_T at $\dot{\gamma} = 208$ sec^{-1} was greatest for the pure SS cell suspension (the uppermost curve in Fig. 1, top) and least for the pure AA cell suspension (the lowest curve in Fig. 1, top). Accordingly, when SS cells were mixed with AA cells, the viscosity fell between these limits. Deoxygenation to a PO2 of 37 torr elevated the viscosity curves for suspensions containing SS cells, especially at high H_T (Fig. 1, bottom). A cross-plot of viscosity (at PO2 = 37 torr) against H_S:H_T ratios at various levels of H_T showed an approximately linear relationship at each H_T (Fig. 2); the slope of the curve became steeper with rising

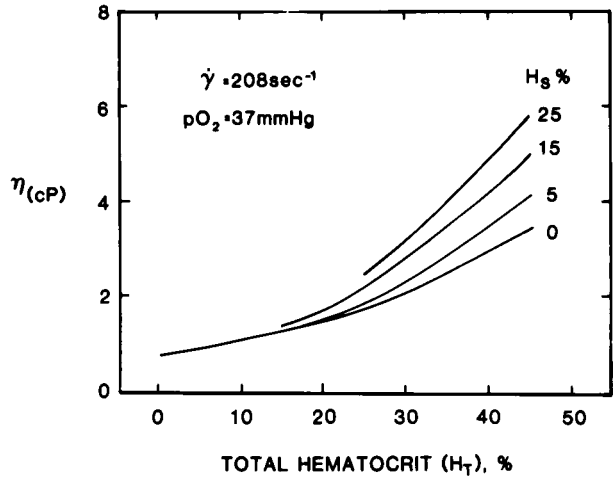

FIG. 3. The data of Figure 1 (*bottom*) replotted to show the rise in viscosity (η) with H_T at various hematocrit levels of sickle cells (H_S). The lines are drawn from points on the least-squares regression lines of Figure 2. These lines are less steep than those in Figure 2.

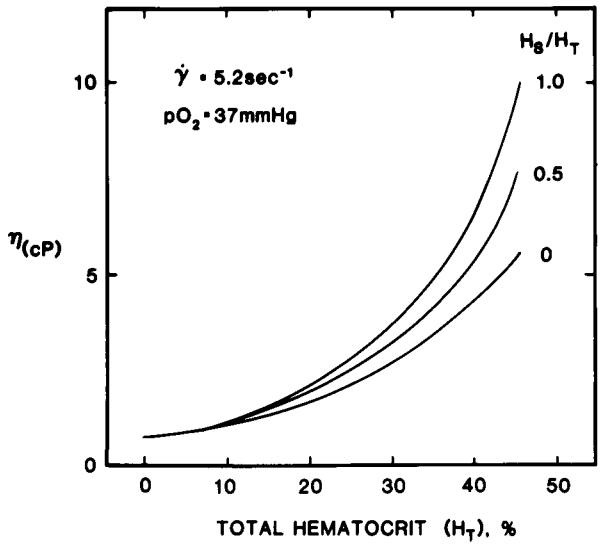

FIG. 4. Viscosities (η) of the same suspensions as Figure 1 (*top*), measured at a lower shear rate ($\dot{\gamma} = 5.2$ sec^{-1}).

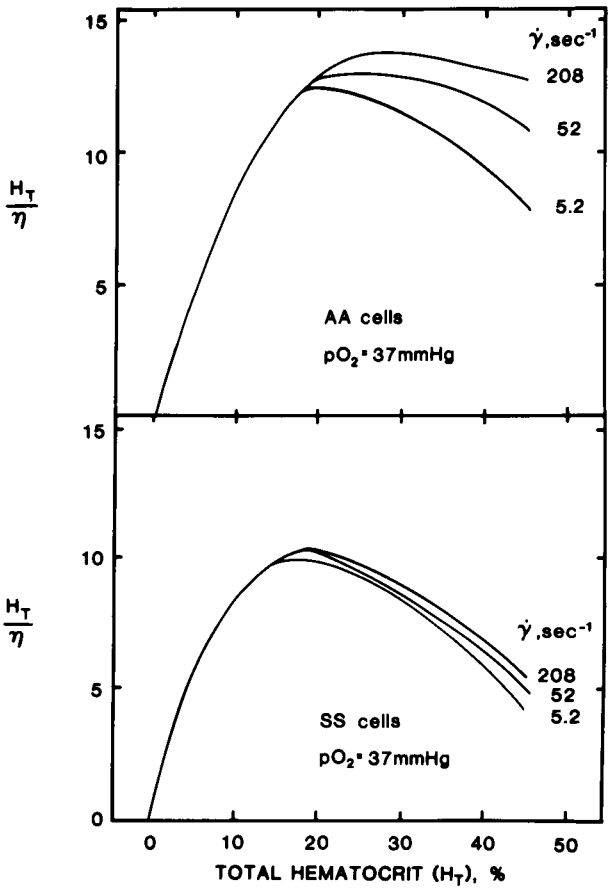

FIG. 5. H_T:η, an estimate of the effectiveness of blood in delivering oxygen, describes a roughly parabolic curve when plotted against H_T. With a decrease in shear rate, the maximum of the parabola becomes lower and shifts to a lower H_T value. Reduced cellular deformability due to the presence of deoxygenated cells with SS hemoglobin lowers the H_T:η curve and the H_T value at which the H_T:η is maximal; shear rate has less effect here. *Top:* Suspensions of normal red cells (hemoglobin AA). *Bottom:* suspensions of sickle red cells.

H_T. The rise in viscosity with H_S:H_T ratio at a constant H_T is attributable to the increasing number of the less deformable SS cells. From the least-squares regression line obtained from the data points in Figure 2, we can also plot the viscosity against H_T, holding H_S constant (Fig. 3). These curves reflect the rise in viscosity resulting from the addition of AA cells to a fixed H_S.

The viscosity of deoxygenated cell suspensions measured at lower shear rates also rose with increased H_S:H_T ratio (Fig. 4); the viscosity values were higher and the slopes steeper than at the higher shear rates. With the reduction in shear rate, the deoxygenated suspension of SS cells increased its viscosity less than the AA preparation did: at $H_T = 45$ percent, the ratio of the viscosities of SS to AA cell suspensions was 2.20 at $\dot{\gamma} = 208$ sec^{-1} and 1.82 at 5.2 sec^{-1}.

The relative effectiveness of a sample of blood in delivering oxygen can be estimated from the ratio of hematocrit value to viscosity (H_T:η).[7] For AA cells, the H_T:η versus H_T plots at various shear rates showed that H_T:η was lower and the change with H_T more marked at lower shear rates (Fig. 5, top). The maximum point of the curve, which reflects the optimal H_T for oxygen delivery, occurred at a lower H_T for the lower shear rates. In comparison to the results on AA cells, for SS cells the curves were lower, showed less variation with shear rate, and had a lower optimal H_T

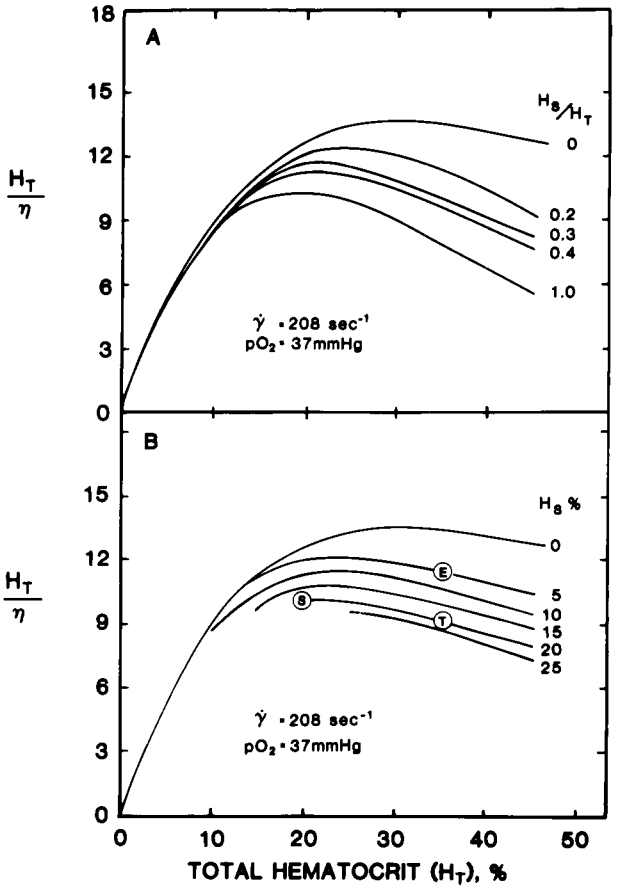

FIG. 6. The H_T:η ratio for suspensions containing mixtures of AA and SS cells. *Top:* Increasing H_S:H_T ratios causes a lowering of the H_T:η curve and a decrease of the H_T value corresponding to the maximal H_T:η ratio. *Bottom:* The same data plotted with constant levels of H_S. Increasing H_T causes a lesser decrease of the H_T:η ratio at constant H_S (*bottom*) than at a constant H_S:H_T ratio (*top*).

(Fig. 5, bottom). The H_T:η ratio of mixtures of SS and AA cells at a PO2 of 37 torr and a shear rate of 208 sec^{-1} were plotted against H_T for a family of H_S:H_T (Fig. 6, top) and H_S (Fig. 6, bottom) values. The AA cell suspensions (H_S:H_T and $H_S = 0$) showed a broad maximum at $H_T \simeq 30$ percent. As H_S:H_T or H_S increased, the height of this maximal H_T:η ratio was reduced and the fall of H_T:η values with rising H_T became steeper. In Figure 7, the diminishing effect of H_S on H_T:η is shown at various H_T values. The H_T:η ratio provided by pure sickle cell suspensions at a usual H_T of 20 percent is indicated by the dashed line. To match this H_T level, a suspension with an H_T of 45 percent must contain less than 10 percent H_S. The shape of the curves indicates that the maximal rate of improvement of H_T:η ratio occurs when the H_S becomes very low.

Discussion

The viscosity of a cell suspension is determined by the concentration of cells, the deformability and aggregation of those cells, and the viscosity of the suspending medium.[10] These factors govern the rheologic behavior of blood flow, not only in the large vessels but also in the microcirculation, which is the principal site of circulatory disturbance in sickle cell disease. The hematocrit value and the viscosity in the microcirculation are both lower than in the larger vessels, but the relationship of the hematocrit level to viscosity in 20- to 30-μm arterioles is the same as that measured in a rotational viscosimeter.[11] It has been shown that in artificial tubes varying from 30 to 200 μm in diameter the H_T:η ratio for hardened cells relative to normal cells does not change.[12] Thus, comparison of SS to AA cells in terms of the relationship of H_T to the viscosity measured in the viscosimeter is probably relevant to the situation in the microcirculation.

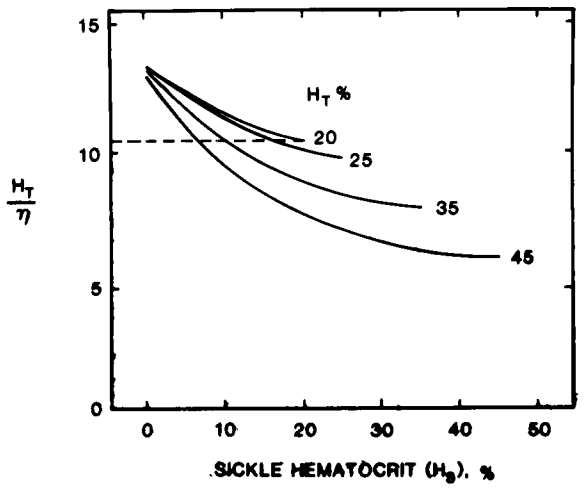

FIG. 7. The H_T:η ratio for the same suspensions as Figure 6. With increasing H_S, the H_T:η ratio falls abruptly, then more gradually, illustrating the rheologic importance of small residual amounts of sickle cells. The dashed line marks the level of the H_T:η ratio provided by sickle cells alone at a H_T value of 20 percent.

The current study was performed on suspensions of red cells in Ringer's solution rather than in plasma, the suspending medium in vivo, in order to focus on the roles of red cell deformability and hematocrit level by eliminating the effects of variations in plasma viscosity and plasma-induced red cell aggregation among the blood samples.[10] The general conclusions attained in the current study, however, should still be applicable to considerations of blood viscosity, especially at high shear rates when rouleaux are dispersed.

The viscosity of a SS cell suspension rises progressively with deoxygenation beginning around 80 torr,[9] and the PO2 level in this study (37 torr) is a point on this continuum. The mixed venous PO2 level in patients with sickle cell disease has been reported to range between 25 and 45 torr;[1] in particular capillaries this value could fall even lower. Whatever the actual PO2 level in the microcirculation, the trends of the relationship of viscosity to H_T, H_S, and the H_S:H_T ratio described here will probably apply.

At full oxygenation, the viscosity of SS cell suspensions is already higher than that of AA cell suspensions.[13] In addition, the effect of deoxygenation on the rheologic behavior of blood should be considered even on the arterial side of the microcirculation. The arterial PO2 level in the patient with sickle cell disease is usually approximately 85 torr, which is less than that in the person with normal cells.[14] At such PO2 levels, polymerization of hemoglobin S can be detected by nuclear magnetic resonance spectroscopy in cells with high mean corpuscular hemoglobin concentrations.[15] The release of oxygen from red cells occurs already in the arterioles,[16] especially when the tissue is relatively hypoxic. Thus, the red cells that enter the capillary bed, where the need for deformability is greatest, may be less fully oxygenated than estimated from arterial oxygen measurements, and hence the viscosity of the blood may be correspondingly higher. If there is stasis, there would be even greater deoxygenation and polymerization before the cells enter the narrowest part of the circulation.

Our analysis addresses only the delivery of blood to the microvasculature, not delivery to the tissues, where oxygen unloading is also determined by the decrease in oxygen affinity known to occur in sickle cells.[17] Modeling of the microcirculation suggests that although this phenomenon helps to normalize capillary PO2, it also tends to increase capillary resistance and reduce blood flow.[18]

In a mixture of SS and AA red cells, when H_T remains constant, the viscosity rises with increasing H_S:H_T ratios (Fig. 2). The relationship between viscosity and the H_S:H_T ratio is approximately linear at a

constant H_T. In experiments with mixtures of normal AA cells and AA cells hardened with glutaraldehyde, Carr and Cokelet[19] also found a rise in viscosity with an increase in the proportion of hardened cells, but the relationship was not linear. The difference between the findings of the two studies may be related to the difference in the degree of cell rigidity: the deoxygenated sickle cells in the current investigation are still somewhat deformable, whereas the glutaraldehyde-hardened cells studied by Carr and Cokelet[19] were not deformable.

Shear rates in the microcirculation are normally considerably higher than 1000 sec^{-1}, and the wall shear stress is on the order of 20 to 60 dynes per cm^2.[11] Above a $\dot{\gamma}$ value of 100 sec^{-1}, neither normal nor sickle cells show any further decreases in viscosity in a viscosimeter, suggesting that a shear stress of approximately 5 dynes per cm^2 has induced maximal deformation in the cell under these circumstances. At a $\dot{\gamma}$ value of 208 sec^{-1}, the relative behavior of sickle and normal cells is similar to that obtained under the shear conditions found in the microcirculation. That measurement is therefore relevant to the physiologic conditions. Our data (Fig. 5) agree with previous observations that the viscosity of suspensions of hardened normal cells[13] and deoxygenated sickle cells[20] does not alter with shear rate as much as it does in normal cells because the harder cells do not change shape easily in response to increasing stress to facilitate fluid motion. As illustrated by Figures 4 and 5, the difference between the viscosities of AA and SS cell suspensions diminishes at lower shear rates. In stasis, where the flow and thus the shear rate is lower, the detrimental rheologic aspects of increasing H_T, even with AA cells, may be magnified.

When a patient receives a simple transfusion, the increase in H_T with a constant H_S leads to a rise in viscosity, which limits the improvement of oxygen delivery despite the increased oxygen carrying capacity. If the patient receives an exchange transfusion with H_T held constant, the fall in H_S will lead to a decrease in viscosity and an improvement in oxygen delivery. The relative advantage of the two types of transfusion can be analyzed by using the curves for H_T:η (Fig. 6, bottom) to illustrate a hypothetical patient. In Figure 6, bottom, the data point S represents a patient with sickle cell disease with $H_T = H_S =$ 20 percent before transfusion treatment. After simple transfusion to raise the H_T to 35 percent, the data point moves along the same H_T:η curve ($H_S = 20\%$) to point T, and there is a drop of about 11 percent in oxygen delivery (H_T:η). However, if an exchange transfusion is carried out to lower the H_S level of the patient to 5 percent, although the H_T is raised to 35 percent with AA cells, the result would be at point E, where the H_T:η ratio is 11 percent greater than at point S and about 23 percent greater than at point T.

An exchange transfusion uses more blood than a simple transfusion. To raise the patient from point S to point T by simple transfusion would require approximately 20 ml per kg of packed cells. An exchange transfusion to bring the patient closer to point E would require 80 ml per kg or more of whole blood, usually incurring exposure to more donors. Iron load would be less with this technique, since a balancing amount of blood is removed. However, the chance of infection or alloimmunization, serious concerns in transfusion, would be increased. Clearly, risk factors should be weighed in planning any therapeutic procedure.

The clinical guidelines for transfusion should be developed considering not only the H_S:H_T ratio but also the absolute levels of H_S and H_T. Since viscosity varies less with H_T when H_S rather than the H_S:H_T ratio is held constant, as shown by comparing Figures 1 and 3, H_S provides a more useful point of reference than the more commonly used H_S:H_T ratio. Transfusion programs that prescribe a maximum H_T of 35 percent and a maximum H_S:H_T ratio of 30 percent, would translate to a maximum H_S of 10.5 percent. Allowing the H_T to rise to 40 percent by simple transfusion would cause the H_S:H_T ratio to fall, but the viscosity would increase and the rheologic aspects would become less favorable. The results of the current study suggest that H_S should be decreased concomitantly to maximize the effectiveness of oxygen transport.

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