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Red Blood Cell Population Dynamics

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INTRODUCTION

The purpose of clinical hematology analyzers is to characterize the health of the patient's hematologic system. These instruments have also supported significant advances in our basic understanding of human physiology. The practical characterization of health by a hematology analyzer is limited both by technical challenges preventing more accurate measurements and by gaps in our understanding of pathophysiology, which compromise our ability to translate measurements into clinical action. The modern hematology analyzer represents a great advance in technical capabilities, providing high-throughput and high-resolution measurements of single red blood cell (RBC) characteristics for tens of thousands of cells.¹

Quantifying Red Cell Dynamics Would Complement the Static Complete Blood Count

The traditional complete blood count (CBC) indices derived from these sophisticated measurements provide a detailed assessment of the current state of a patient's hematologic system: assessment of the total oxygen carrying capacity by the hematocrit (HCT) or hemoglobin (HGB) levels, measurement of the RBC mean corpuscular volume (MCV), mean corpuscular Hb (MCH), mean corpuscular Hb concentration (MCHC), and an assessment of the current magnitude of variation in volume from 1 RBC to the next (red cell distribution width [RDW]). This multivariate characterization of the current state of the hematologic system is relevant to the screening, diagnosis, and monitoring of almost all diseases. However, the circulating RBC population is constantly changing, with about 2,000,000 new RBCs entering from the bone marrow every second and about the same number being cleared and recycled. Knowledge of the rates of change in the circulating RBC population would provide complementary information on the patient's hematologic

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system, supporting more accurate inference of the prior states of the hematologic system and its likely future states.

Reticulocyte Counts Provide a Glimpse of Value of Red Cell Dynamics

Automated reticulocyte counts demonstrate the clinical usefulness of an assessment of dynamic aspects of the hematologic system.^{2,3} The automated reticulocyte count enables estimation of the rate of RBC production, and that estimate can help distinguish between anemias with different cause, for instance, distinguishing those resulting from productive deficits from those resulting from hemolysis or other destructive processes.

Current clinical use of the automated reticulocyte count also provides an example of the added value obtained by combining high-resolution measurements with quantitative models of physiology. In particular, more subtle diagnostic distinctions and prognostications can be made by combining this new quantification of a physiologic process (ie, the reticulocyte count) with traditional CBC indices like HCT or HGB, which assess the size of the current circulating RBC population. Based on our knowledge of physiologic homeostatic mechanisms, we expect a mild anemia to trigger a compensatory increase in reticulocyte production, and we would expect a more significant anemia to trigger a more significant increase in reticulocyte production. The reticulocyte count enables us to determine not only whether reticulocyte production is qualitatively increased in the presence of anemia but also whether the magnitude of increase in production is appropriate. Calculations such as the corrected reticulocyte count are examples of mechanistic model-based inference from clinical laboratory measurements. Using our model of the quantitative physiologic relationship between RBC production rate and anemia, the magnitude of a reticulocyte count can be compared with the magnitude of the anemia and an assessment can be made as to whether the patient's physiologic systems are responding in a healthy fashion. It is thus possible to identify situations that might qualitatively seem to be healthy. For instance, if there is a decreased HCT or HGB level, and the reticulocyte count is increased, the qualitative assessment would be that the patient's response was appropriate and that the anemia was not caused by a deficit in production. The corrected reticulocyte count or the reticulocyte production index are model-based calculations, which provide a more nuanced assessment of erythropoietic output and enable more precise diagnostic interpretations.^{4,5} Although the automated reticulocyte count represents a significant technical advance, with proven clinical value, the measurement itself is imprecise, with repeat measurements of the same blood sample varying by more than 10%, and repeat measurements in the same healthy person varying by as much as 30%.^{6,7} The fact that such a crude estimate of RBC production rate is so useful underscores the potential clinical value of other, even crude, assessments of RBC population dynamics.

Estimating Red Cell Maturation and Clearance Rates Requires Modeling

The reticulocyte count provides an assessment of the RBC production rate, but there are no corresponding ways to assess the rate of RBC maturation that occurs during an RBC's ~110-day life span in the circulation, nor is there a way to assess the rate of RBC clearance. Other clinical laboratory measurements such as bilirubin and haptoglobin provide some vague information on RBC turnover, but they are of limited value. RBC turnover is difficult to

study.^{8–10} Methods have been developed and usually involve labeling, reinfusion, and serial sampling of blood. Other techniques using exhaled carbon monoxide are desirable, in that they are less invasive and do not require weeks of preparation and multiple blood samples, but they have other limitations, including poor precision. A better understanding of RBC life span variation and control may suggest better markers of RBC age, which would enable easier determination of RBC age. In the meantime, mathematical models can be used to provide an initial estimate of RBC maturation and turnover. Without a model of RBC aging, RBC clearance cannot be measured noninvasively. Single RBCs would have to be tracked over many weeks up to the time of clearance and beyond, something which is not currently feasible. Estimates of RBC maturation and clearance therefore require modeling but, like automated reticulocyte counts, are likely, even if crude, to provide new insights into physiology¹¹ and new diagnostic applications.¹²

Modern hematology analyzers provide sophisticated and high-throughput data sets, which present a great opportunity to improve characterization of hematologic health dramatically. In particular, these high-resolution population measurements provide sufficient data to support inferences about RBC population dynamics: how quickly RBC characteristics are changing in the circulating population, and how much those rates vary from 1 RBC to the next and for 1 RBC over time. There are many measurable characteristics of RBCs, and many of them change over time. A study of RBC population dynamics may thus focus on many different cellular characteristics and how they change over time. RBC volume and RBC Hb content are 2 characteristics of known clinical importance, which can be measured accurately and with high throughput. The population dynamics of RBC volume and RBC Hb content, and the rate of clearance of RBCs, are therefore the focus of the rest of this article, in which:

- Current understanding of RBC dynamics from basic scientific studies is summarized
- A conceptual model of RBC population dynamics is synthesized, and a method to infer RBC population dynamics from hematology analyzer measurements is described
- Current and future opportunities for measuring dynamic features of blood cell populations and their potential applications are reviewed

BASIC SCIENTIFIC STUDIES SHOW ASPECTS OF RED BLOOD CELL POPULATION DYNAMICS

The RBC population is in constant flux. About 2 million cells enter the bloodstream from the bone marrow of a typical healthy human adult every second, with about the same number being cleared. The typical RBC circulates for 100 to 120 days, before being recycled and replaced. Basic science studies have shown that many characteristics change during the life span of the RBCs in the peripheral circulation¹³: volume decreases by about 20%,^{14–16} Hb mass decreases by about 15%,^{14,15} surface area is reduced, surface/volume ratio increases, microvesicles form and are shed, phosphatidyl-serine symmetry changes,

and more. Many more changes occur to individual RBCs in specific contexts, and the physiologic basis and implications are not understood for most of them.

Single-RBC measurements of volume provide richer information on in vivo hematologic processes than just the population averages. The combination of automated reticulocyte identification with these single-cell measurements provides this level of detail in an age-stratified way. Some clinical instruments, including the Siemens Advia 120, 2120, and 2120i (Siemens Healthcare Diagnostics, Tarrytown, NY), and the Abbott Cell-DYN Sapphire (Abbott Laboratories, Santa Clara, CA), provide simultaneous measurement of single-RBC volume, single-RBC Hb concentration, and a reticulocyte dye concentration. These measurements provide high-resolution multidimensional characterization (volume, Hb concentration, reticulocyte status) of the full circulating RBC population and the reticulocyte population. Signatures of the in vivo single-RBC dynamics can be seen in the nature and magnitude of variation in each quantity, for instance the single-RBC volume distribution as shown in Fig. 1 represents the net effects of these processes on the volumes of all circulating RBCs.

Empirical Studies of In Vivo Volume Dynamics

Young RBCs have long been known to be larger than mature or senescent cells. With the advent of automated reticulocyte counts,^{2,3,13} it became possible to quantify the volume differences between young RBCs and those that are more mature. There are many open questions regarding the molecular or cellular processes that regulate these changes in volume, but with high resolution measurement of RBC volume distributions, the changes can be quantified and hypotheses tested regarding mechanisms.

Initial volume changes are fast—Several studies have found that the rate of volume change for young RBCs is rapid, with at least half of the total volume change of the RBCs likely occurring during the first week in the circulation.^{15,17,18}

Subsequent volume changes are slow—Experimental studies have also found that the size of each RBC continues to decrease, albeit more slowly, after this first rapid phase occurring early in the lifetime of the RBC in the circulation.^{14,16}

Empirical Studies of In Vivo Hemoglobin Content Dynamics

Some modern hematology analyzers measure single-RBC Hb concentration simultaneously with single-RBC volume.¹ Although Hb concentration is the characteristic directly reported by these instruments it is conceptually simpler to work with single-RBC Hb mass or content instead of Hb concentration because simultaneous changes in RBC volume complicate the interpretation. By multiplying the single-RBC Hb concentration by the volume measurement of the same RBC, it is straightforward to derive the Hb mass, with the caveat that Hb mass is derived from two instrument-reported values and may be less accurate than each reported value individually and may also be biased by any correlation in the errors of the 2 values.

The RBC mass is largely determined by the Hb mass, and the RBC density is therefore largely determined by the Hb concentration. It has been challenging to identify physiologic

markers of RBC age, and some initial studies suggested that RBC density increased monotonically with age, but because of the strong correlation between RBC density and Hb concentration, this assumption implies that RBC Hb concentration increased monotonically with age. More detailed studies in recent years have resolved some of this confusion and present a picture of Hb content dynamics that is qualitatively similar to that for single-RBC volume.¹⁴

Initial hemoglobin changes are more significant—RBC density, and thus RBC Hb concentration, seem to increase during the first week or so after a reticulocyte has entered the circulation.¹⁴ Because RBC volume is decreasing during this time as well, a net increase in Hb concentration requires that the Hb content must not decrease as much.¹⁵

Subsequent hemoglobin changes are less pronounced—Inferences about subsequent changes in RBC Hb content can be made from measurements of RBC volume and Hb concentration. After the first week or so in circulation, RBC density and Hb concentration do not change markedly.^{14,16,19} Recent independent measurements with extremely high precision support the conclusion that there is little variation in RBC density across the entire cell population, which represents cells of all ages.¹⁹

Overall, it seems that for a single reticulocyte entering the peripheral circulation from the bone marrow, the volume changes by about 10% in the first week or so, and the Hb content decreases by about 7%. The volume and Hb content of the single cell then continue to decrease more slowly over the remaining ~3 months of the life span of the RBC, with the total reductions after the first week being comparable to those occurring during the first week, but at slower rates. These volume and Hb content changes are regulated such that there is little variation in the single-RBC Hb concentration from 1 cell to the next.

QUANTIFICATION OF RED BLOOD CELL POPULATION DYNAMICS IN THE CLINICAL LABORATORY

A conceptual and mathematical model can be derived for RBC volume and Hb dynamics from these basic science studies. The model can then be combined with the high-resolution measurements available on some modern hematology analyzers to quantify RBC population dynamics for individual patients. Fig. 2 shows the volume and Hb distributions measured for an individual healthy patient on an Abbott Cell-DYN Sapphire analyzer. These high-resolution data sets provide additional information on the dynamic processes controlling reticulocyte and RBC maturation. One can begin with the empirical observations described earlier and combine them with these high-resolution data sets to constrain the range of models of RBC dynamics sufficiently that these dynamics can be quantified for individual patients.

Deriving a Model of Red Blood Cell Population Dynamics from Basic Science and Hematology Analyzer Data

The volume of a single RBC changes during its time in the circulation, and the Hb content changes as well. There is strong evidence suggesting that these rates of change are initially

relatively fast and subsequently relatively slow. These empirical findings from basic science studies provide constraints on the poorly understood processes responsible for RBC maturation in the peripheral circulation, allowing exclusion of several possible explanations and a focus on a more quantitative description of these processes, even in the absence of any detailed molecular characterization. For instance, Fig. 3 shows the maturation of a single reticulocyte based on these basic science studies. A model of RBC population dynamics would describe the path through the volume-Hb plane that a young reticulocyte is most likely to take during its lifetime.

There are an infinite number of possible trajectories, but many of them can be excluded, because they contradict the empirical results described earlier. For instance, the top trajectory in Fig. 3 requires an initial increase in the Hb content of the RBC, which is contradicted by basic science studies and available empirical data. Any model of RBC population dynamics that requires such a trajectory can thus be excluded. Using the high-resolution data from the hematology analyzer in Fig. 2, the bottom trajectory in Fig. 3 can also be excluded, because there are no cells in a region of the plane through which many reticulocytes would have to pass according to models requiring this sort of trajectory. The middle trajectory is therefore the most plausible.

To paraphrase the statistician George Box, all mathematical models are wrong, but some are useful. Any model of RBC volume and Hb dynamics is therefore expected to be wrong in some ways, but if it captures enough of the major features of the underlying pathophysiologic processes, the estimates it provides of these true physiologic processes are good enough to be useful in helping us understand dynamic aspects of physiology and useful in complementing traditional diagnostic methods as will be discussed below.

An Example Model of Red Blood Cell Population Dynamics

The range of possible models is strongly constrained by basic scientific studies, some of which were reviewed earlier, and by high-resolution hematology analyzer measurements, such as those shown in Fig. 2, but it is still possible to propose different models that are consistent with these studies and data. Informed guesses must then be made, which will certainly not be entirely accurate, but the model can then be tested with new data, its predictions compared, and we can decide if we feel confident that the model is sufficiently accurate to be useful. At least 1 published model has satisfied this sort of validation, and its basic features and assumptions are reviewed, as shown in Fig. 4¹²:

- Single-RBC volume and Hb content decrease rapidly until the Hb concentration of the RBC approaches the population mean (MCHC).
- Single-RBC volume and Hb then decrease steadily and more slowly.
- There are small fluctuations in the rates of change in volume and Hb content for individual cells.
- The probability of clearance for a particular RBC can be approximated as a threshold function of its volume and Hb content.

The model describes the rate of volume change for a particular RBC as a function of its current volume and current Hb content.¹² The function has a mathematical form which reflects the 2 phases of volume reduction found by the basic science studies described earlier. The first phase of volume reduction involves faster rates of change and lasts until the Hb concentration of the RBC is close to the population mean (MCHC). The second phase of volume reduction then occurs more slowly. The model includes patient-specific parameters, allowing estimation of rates of volume reduction personalized for each patient, with β_v governing the fast phase of volume change and α governing the slow phase. The model also includes parameters for the Hb dynamics, both the fast (β_h) and slow phases (α), as well as the magnitude of the fluctuations in volume reduction (D_v) and Hb reduction (D_h), and a parameter for the threshold function (v_c) used to approximate the clearance process (Fig. 5).

This model then allows these parameters to be quantified for individual patients, yielding estimates of the rates of different dynamic physiologic processes related to RBC rates and clearance. This novel dynamic information provides new insight into physiology, with potential clinical diagnostic applications.

APPLICATIONS OF RED BLOOD CELL POPULATION DYNAMICS AND FUTURE DIRECTIONS: TOWARD A MORE COMPLETE BLOOD COUNT

The value of high-resolution data from the modern clinical hematology analyzer derives from the physiologic insights it enables and from the diagnostic applications it supports. The example model described earlier provides an unusual opportunity to compare RBC clearance rates in patients by measuring CBCs and reticulocyte counts. This exercise has suggested, among other things, that the RBC clearance process is tightly regulated and may be modulated in pathologic situations.

Red Blood Cell Clearance Is Tightly Regulated

One of the goals of modeling RBC population dynamics is to generate new insight into basic physiology. RBC clearance processes are difficult to study, and we are only now beginning to understand the magnitude of variation in the RBC clearance process among healthy individuals.^{8,9} The clearance rate can be estimated using the model described earlier and shows a coefficient of variation (1.1%) in a healthy population, suggesting that the clearance rate is more tightly controlled than any of the traditional CBC indices (Fig. 6). The model itself, as described in Ref.¹² yielded consistent estimates with a range of functional forms for the volume and Hb dynamics. All of the specific equations were deduced from the same set of empirical constraints, and it is reassuring when the quantitative predictions they enable, such as tight regulation of the clearance process, are robust to the specific functional form. The legitimacy of the estimate of RBC clearance rate rests not on whether the single-RBC Hb and volume dynamics are assumed to be exponential or linear with respect to the current volume or Hb level of an RBC but instead rests only on the knowledge that there is an initial fast phase of volume and Hb reduction followed by a subsequent slow phase, and that the speed of the fast phase is correlated with the difference between the current Hb concentration of the RBC and that of some population-wide target. This enhanced

understanding of basic physiology can then be used to improve our understanding of pathologic situations such as iron deficiency anemia.

Red Blood Cell Clearance Seems to be Delayed in States of Red Blood Cell Production Deficits

Having developed and validated this model, it can be used to estimate the RBC clearance rate for patients and compare their estimated clearance rates with those from healthy individuals to understand any effect these diseases may have on the clearance rate or any adaptive response of the clearance rate to these diseases. Iron deficiency is a common condition compromising erythropoiesis. Iron deficiency anemia is associated with a decreased MCV and often an increased RDW. Modeling of the RBC population dynamics in individuals with mild iron deficiency anemia (Fig. 7) shows that their clearance threshold has been decreased. Because the clearance threshold is expressed as a fraction of the MCV, this decrease in clearance threshold occurs above and beyond the well-known decrease in MCV.

A delay in RBC clearance transiently increases the circulating mass of RBCs. Given that iron deficiency anemia involves a decrease in erythropoietic output, this model-derived observation of delayed clearance suggests a mechanism: perhaps RBC production decreases slightly as a result of an incipient iron deficiency, and this decreased production triggers compensatory delay in clearance to maintain circulating red cell mass. This hypothesis is shown in Fig. 8.

Dynamic modeling of red cell populations in patients with iron deficiency anemia thus suggests that the RBC clearance threshold is decreased, perhaps as compensation for diminished output in the face of decreased iron stores. The lowered clearance threshold maintains circulating RBC mass, confounding the diagnosis of the anemia based on decreasing HCT or HGB levels. By using a model of RBC population dynamics, it might be possible to identify the clearance delay directly and predict the anemia before it appears.

Delayed Clearance Predicts Iron Deficiency Anemia

A goal of modeling RBC population dynamics is the discovery of novel diagnostic approaches. The model discussed earlier suggests that (1) RBC clearance is tightly regulated in healthy people, (2) it is delayed in the early phases of iron deficiency before anemia has developed, and (3) the clearance rate can be estimated as a threshold function (v_c). It may then be possible to develop an early warning biomarker for iron deficiency anemia by measuring v_c or some other estimate of RBC clearance. Previous studies¹² have found that it is possible to identify cases of iron deficiency anemia in people with normal CBCs using this approach up to 90 days before anemia is discovered, with sensitivity greater than 50% and specificity greater than 90%. Because these people all have normal CBCs, sensitivity of detection using traditional CBC indices alone is 0%.¹² This high sensitivity and specificity for iron deficiency anemia suggest that the timescale for homeostatic response to iron deficient states is sufficiently long (multiple months) that this approach may provide an opportunity to improve screening programs for iron deficiency in adults at risk of

gastrointestinal malignancy and in young children at risk of cognitive deficits from nutritional deficiency.

Future Directions

The example model presented in this article characterizes RBC population dynamics using 6 parameters, which describe patient-specific estimates of the fast and slow phases of volume and Hb reduction, the magnitude of their fluctuations, and the clearance rate as estimated by a threshold function. The model allows estimation of these dynamic parameters for individual patients using existing CBC and reticulocyte measurements, thereby providing several new insights into RBC physiology. This article focuses on just one of these insights, namely the tight regulation of RBC clearance rate and its modulation in iron deficiency anemia. The other model parameters provide additional insights into RBC physiology, which complement existing knowledge and present additional opportunities for diagnostic applications. Other models of RBC population dynamics, particularly those that are more accurate because they integrate new understanding, will provide additional and more powerful insights.

Modern hematology analyzers provide high-resolution and high-throughput characterization not just of RBC populations but also of white blood cell lineages and platelets. The characterization of white blood cell lineages and subtypes has become particularly sophisticated in recent years. Models of the population dynamics of these other cell types will likely show important details of physiology, with even greater diagnostic possibilities.

The power of dynamic population models of RBCs depends on the richness of the measurements made by the hematology analyzer. The combined optical measurement of RBC volume and Hb level developed a few decades ago was an important technological step.¹ It is concerning that the availability of instruments with this enhanced technical sophistication may be decreasing as clinical laboratories prioritize features such as automation over the richness of the measurements. This sort of regressive step in terms of technical capability in the clinical laboratory is worrisome for the future role of the clinical laboratory in diagnostic medicine. Almost every other area of medical care has embraced the potential of large data sets to inform personalized and precision medicine. Many hospital departments are collecting vast databases of high-resolution measurements: genetic, proteomic, metabolomic, transcriptomic, cytometric, and more. The current diagnostic purpose of these data sets is not always clear but their future value is unquestioned. If the clinical laboratory does not provide high-resolution CBC data to enable the sort of modeling and analysis described in this article, then other hospital departments will do so. In that case, as large data sets and the expertise to transform them into clinically actionable information become driving forces in modern health care, the clinical laboratory will play a smaller and smaller role. As others have noted,²⁰ the clinical laboratory currently has the expertise and infrastructure to take a leading role, transforming the recent technical advances in molecular and cellular measurement into clinically actionable information.

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Abbreviations

CBC	Complete blood count
Hb	Mass of hemoglobin in a single RBC
HCT	Hematocrit
HGB	Mass of hemoglobin per unit volume blood
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
RBC	Red blood cell count
RDW	Red cell distribution width

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KEY POINTS

- Traditional complete blood count (CBC) indices (eg, hematocrit level, hemoglobin level, mean corpuscular volume) provide a snapshot of the current state of the hematologic system.
- The hematologic system is constantly changing, with about 2 million red blood cells (RBCs) per second entering the circulation from the bone marrow and about the same number being cleared per second.
- Modern hematology analyzers are sophisticated instruments, measuring single-cell characteristics at high resolution and with high throughput.
- These measured distributions of single-cell characteristics can be combined with our knowledge of physiology to make inferences about RBC population dynamics and to quantify physiologic dynamics, like how quickly new RBCs are being produced, how quickly a typical RBC's volume is changing as it matures during its ~110-day life span in the circulation, and how quickly RBCs are being cleared and recycled.
- Information on physiologic dynamics will reveal new details of physiologic mechanisms and offer new diagnostic opportunities for existing CBC measurements.

Fig. 1.

Changes occurring over the course of the life span of an RBC in the circulation. Single-RBC changes in volume and HGB content integrate over the population and over time to determine the variance in volume and Hb content measured in a CBC. Reticulocytes exit from the bone marrow (*top right*). Their volume and Hb content decline over the course of their ~110-day life span. The single-RBC dynamic processes combine to generate the distributions in volume (RDW) and Hb content, which can be measured by some modern hematology analyzers.

Fig. 2.

Single-RBC (*red*) and single-reticulocyte (*blue*) volume and Hb mass distributions. The red contours show the probability density for volume and Hb in the total RBC population of a healthy adult. The red circle shows the MCV, MCH position. The RDW bar shows the extent of the coefficient of variation in volume. There is a thin gray line connecting the MCV, MCH point (*red circle*) to the origin and representing the MCHC. Red contours enclose 90%, 75%, 50%, and 25% percent of cells close to the mean. The blue contours show the same probability density for reticulocytes, with the mean volume and Hb content (rMCV, rMCH) shown as a blue circle. The rRDW bar shows the extent of the coefficient of variation in volume. There is a thin gray line connecting the rMCV, rMCH point to the origin, which represents the average reticulocyte Hb concentration (rMCHC). Blue contours enclose 75%, 50%, and 25% of cells.

Fig. 3.

Trajectories for an individual maturing RBC. The large red circle in the top right represents a reticulocyte sampled from the reticulocyte volume–Hb content distribution (*light blue oval*). The smaller red circle represents a typical mature RBC sampled from the RBC volume–Hb content distribution (*red oval*). The green dashed lines represent 3 hypothetical trajectories. The top trajectory requires an increase in single-RBC Hb content, and any model requiring that sort of trajectory can be excluded based on basic science knowledge. The bottom straight line trajectory can be excluded, because it passes through a space in the plane not covered by either the RBC distribution (*red oval*) or the reticulocyte distribution (*blue oval*). Empirical measurements show that no RBCs of any age are found in these regions, and models requiring this sort of trajectory can be excluded. The middle trajectory is most consistent with basic science findings and hematology instrument measurements.

Fig. 4.

An example model of RBC population dynamics. Reticulocytes enter from the bone marrow. Their volume and Hb content decrease rapidly at first toward the population mean (gray line through the red circle marking the MCV and MCH). The volume and Hb content of a single RBC continue to decrease with small fluctuations until the cell is cleared and recycled, with the probability of clearance approximated by a threshold function of the volume and Hb level of the RBC. A cell with Hb concentration equal to the MCHC is most likely to be cleared when its volume reaches v_c . Cells with higher or lower Hb concentrations are more likely to be cleared at lower or higher volumes, as shown by the clearance threshold line.

Fig. 5. A model of RBC volume and Hb dynamics. Reticulocytes enter from the bone marrow (*top right*). Their volume and Hb content decrease rapidly at first toward the population mean (*center of thick black line* designated as MCHC), with β_v quantifying the rate of volume change and β_h quantifying the rate of Hb change. The volume and Hb content of a single RBC continue to decrease (α) with small fluctuations (D_v and D_h), until the cell is cleared and recycled, with the probability of clearance approximated by a threshold function of the volume and Hb of the RBC. A cell with Hb concentration equal to the MCHC is most likely to be cleared when its volume reaches v_c . Cells with higher or lower Hb concentrations are more likely to be cleared at lower or higher volumes, as shown by the red clearance threshold line. The thick red horizontal arrows show the coefficient of variation in the v_c and the MCV. There is less variation in the estimated clearance threshold.

Parameter	Clearance threshold	HCT	RBC	MCV	RDW	MCH	MCHC	HGB	retic
Coefficient of variation	1.1%	7.3	7.5	4.1	4.5	4.1	2.6	7.9	53.3

Fig. 6.

Variation in traditional and dynamic CBC indices. The estimated clearance threshold (v_c) has a smaller coefficient of variation in 700 healthy individuals than any of the other traditional CBC indices or the reticulocyte count. The clearance threshold therefore has the potential to be a specific marker of disease, because any variation would be easily distinguishable from the narrow normal range.

Fig. 7.

Clearance threshold in healthy individuals and those with iron deficiency anemia (IDA). The clearance threshold is expressed as a fraction of the MCV, with v_c equal to the volume at which an RBC with an Hb concentration equal to the population mean MCHC would be most likely to be cleared. Healthy individuals have a v_c tightly clustered around 80% of the MCV. Individuals with IDA have a significantly lower v_c . These individuals with IDA had v_c s in the normal range before the development of IDA, and a decreased v_c may therefore serve as an early warning sign for impending IDA.

Fig. 8. Hypothesized homeostatic mechanism for RBC clearance delay. The lowered RBC clearance threshold found in patients with decreased erythropoiesis is typical of iron deficiency anemia and suggests that the clearance delay may serve as a temporary compensatory response to decreased RBC production, maintaining RBC mass in the face of decreased production. Left-hand panels show a schematic of the mechanism. Right-hand panels show support for this idea provided by CBC results for an individual when healthy (*top right*), with frank iron deficiency anemia (*bottom right*), and a latent anemia state 2 months before the anemia, when the evidence of clearance delay is shown by the increasing fraction of the RBCs appearing lower than the 85th percentile (*red shaded regions* in each right-hand panel) along the MCHC line.