Importance of Serum Hemoglobin in Hormone Refractory Prostate Cancer

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ABSTRACT

Objectives: **In the search for an early measure of response in hormone refractory prostate cancer (HRPC), most have targeted changes in serum prostate-specific antigen (PSA). Up to this point, no one has targeted changes in serum hemoglobin during treatment. If dynamic changes in hemoglobin after treatment provide additive prognostic information to dynamic changes in PSA, then we should consider and test ways to incorporate serum hemoglobin into measures of response in HRPC.**

Methods: **Our patients consisted of 321 men who were studied on Cancer and Leukemia Group B protocols 9181 and 9182. We fit serial values of PSA and hemoglobin with an exponential model: $y = \exp(\alpha + \beta*t + \gamma*t^2)$ with y symbolizing either PSA or hemoglobin and t denoting time. We then used the Cox proportional hazard model to relate the parameters of the model (α, β, and γ) to subsequent survival.**

Results: **We found that the exponential model fit serial measurements of both PSA and serum hemoglobin well, and all three of the parameters for both markers related closely to subsequent survival ($P \leq 0.003$). The Cox model suggested a composite hazard score (HS) as a way to consolidate the information from serial measurements of both serum markers, and we observed that those with HS < 0 enjoyed a longer survival.**

Conclusion: **Because serial measurements of serum hemoglobin during treatment of HRPC add prognostic information to serial measurements of PSA, we hypothesize that combining the dynamic changes in serum hemoglobin with those of PSA could lead to an improved measure of response in HRPC.**

INTRODUCTION

In the United States, ~30,000 men die each year of HRPC[2] (1), and for this stage of prostate cancer, few therapies have prolonged survival (2). Thus, there has been considerable interest in testing new therapies in men with HRPC and, coupled with this, an equal interest in using PSA for an early measure of response. For example, a group at NIH recently convened a Prostate-Specific Antigen Working Group to form consensus definitions of PSA progression and response after treatment of HRPC (3). It was their hope that the agreed on definitions would allow investigators to quickly identify therapies that are active in Phase I and II studies of HRPC and that such studies would lead to eventual confirmation by prospective Phase III trials. To learn more about PSA in HRPC, we previously studied the collected serial measurements of PSA in men on two completed CALGB studies (9181 and 9182) and found that the combination of the natural logarithm of PSA and the relative velocity of PSA were both important for subsequent survival (4, 5). Furthermore, we learned that the natural logarithms (symbolized throughout this paper as "log") of serum hemoglobin and patient weight were two factors that added prognostic information (5). Although patient weight seemed to be a late operating factor, *i.e.*, one that is important at a time near death, serum hemoglobin, like PSA, seemed to be important for prognosis at early as well as late times in follow-up.[3] Thus, we decided to study more closely the dynamics of both PSA and serum hemoglobin during the period of treatment of HRPC. Finally, in hope of developing tools that could be useful for modeling the pattern of change in both of these serum markers after treatment, we explored the use of a parametric exponential function to fit serial measurements of either PSA or hemoglobin over time. We reasoned that if such a function could accurately fit the trends in PSA and hemoglobin with time, then its parameters might efficiently summarize many serial measurements. If the fit of the function was good and its parameters closely related to survival, then such parameters might provide a tool for measuring response in HRPC.

PATIENTS AND METHODS

Our study population consisted of men enrolled in the same two CALGB studies used in our previous report (5).

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[2] The abbreviations used are: HRPC, hormone refractory prostate cancer; PSA, prostate-specific antigen; CALGB, Cancer and Leukemia Group B; HS, hazard score.

[3] Unpublished observations.

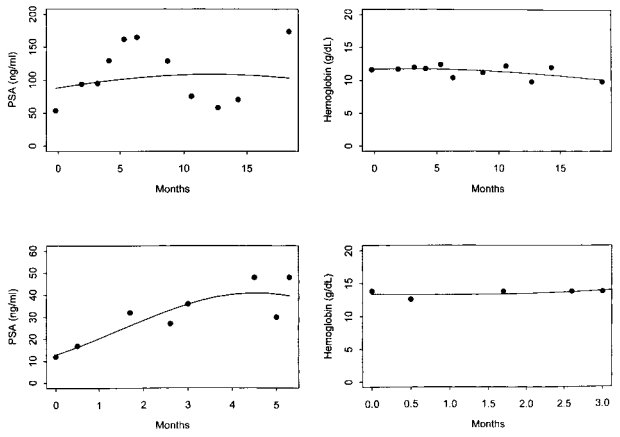

Fig. 1 Composite of four plots of observed values (*points*) of PSA (*left plots*) or serum hemoglobin (*right plots*) *versus* time of follow-up in months. The lines are the fits provided by the model of Eq. A. The upper two plots are for one patient, and the lower two for a second patient.

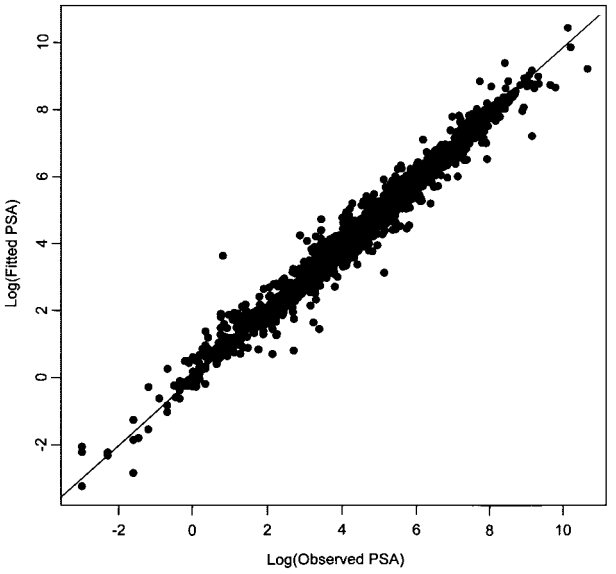

Fig. 2 Plot of natural logarithm of fitted PSA (ng/ml) on vertical axis *versus* logarithm of observed PSA on the horizontal axis. The fitted values come from Eq. A, and the line shows the points at which perfect fit occurs.

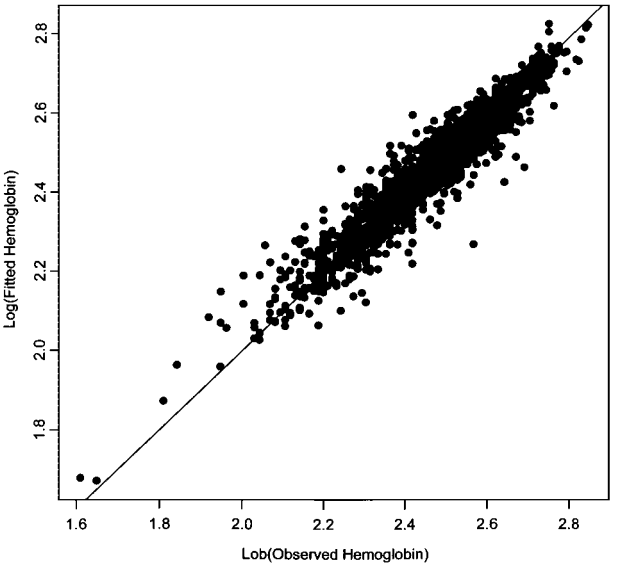

Fig. 3 Plot of natural logarithm of fitted serum hemoglobin (gm/dl) on vertical axis *versus* logarithm of observed serum hemoglobin on the horizontal axis. The fitted values come from Eq. A, and the line shows the points at which perfect fit occurs.

The main results regarding treatment have also been published previously (6, 7). Because the exponential function required at least three serial values of PSA and hemoglobin and because neither of these studies mandated serial measurements of PSA and hemoglobin, some of the original study patients were necessarily excluded. Furthermore, a few had participated in both studies. Thus, the number of unique patients available for our study of serial values of PSA and hemoglobin was a subset of 321 of the original 391. Of these, 297 were followed to death, and the remaining 24 were considered censored at the last time of follow-up. CALGB protocol 9181 evaluated the effect of two doses of megestrol acetate in HRPC, and CALGB protocol 9182 evaluated the effect of mitoxantrone plus hydrocortisone *versus* hydrocortisone alone in HRPC. Neither study found that the treatments affected survival (6, 7). Men on both studies provided signed, informed consent, and both protocols were approved by each CALGB institution's review board.

Model Used to Fit Serial Values of PSA and Hemoglobin after Treatment. Because we have found that PSA is a more important prognostic variable when used as a natural logarithm (4, 5, 8), and because we also found this true for serum hemoglobin (5), we chose to model log(PSA) and log(hemoglobin) rather than their respective untransformed values. Furthermore, because the simplest set of equations to begin with are the polynomials, we used a second order polynomial, *i.e.*, one that includes first and second derivatives with respect to time. Thus, if the value of the serum marker (either PSA or hemoglobin) is symbolized at time t as y, then the model we used is given by the following equation:

$$y = \exp(\alpha + \beta * t + \gamma * t^2) \qquad \text{(A)}$$

Parameter α relates to the marker value at the beginning of study; parameter γ relates to the second derivative of log-(marker) with respect to time; and parameters β and γ together relate to the first derivative. Because time, t, was measured in units of months, the units of β were 1/months, and the units of γ were 1/months^2. This formulation implies that the relative velocity (rv) of PSA relates to both β and γ. Specifically,

$$rv = \left(\frac{dy/dt}{y}\right) = \beta + 2 * \gamma * t \qquad \text{(B)}$$

Thus, if γ is not equal to zero, then this model implies that the rv will change with time. We used a least-squares algorithm to

Table 1 Model parameters obtained from least-squares fit of serial measurements of log(PSA) and log(hemoglobin) in HRPC

	PSA		Hemoglobin	
Parameter	Mean	Range	Mean	Range
α	4.68	−0.15–9.4	2.49	2.1–2.8
β	0.050	−2.3–1.5	−0.0067	−0.38–0.43
γ	0.017	−0.61–0.57	−0.0030	−0.25–0.001

fit the data with this model (9), and to avoid unnatural rises and falls in the fit and instead to capture a smoothed trend in the data, we restricted the value of γ to zero when there were fewer than four serial values.

Statistical Methods. To examine the relationship between survival time and the model parameters, we used the Cox proportional hazard model (10, 11), and for this, we used the interval method of recording survival time with the time interval beginning at the time of last serum sample and ending with either death or the time of censoring. We examined the fit of the final Cox model fit in two ways. We used the method of Grambsch and Therneau to look for trends in the model's coefficient values with time (12, 13), and we used the method suggested by Hosmer and Lemeshow (11) to compare observed events with expected events obtained from the final Cox model and over separate deciles of risk score. We also used ANOVA and linear regression techniques when indicated. All *P*s are for two-sided tests of hypothesis, and all analyses were done with the S-PLUS software package (MathSoft, Inc., Seattle, WA).

RESULTS

Overall Fit of the Exponential Model to the Data. Fig. 1 provides an example of how the model fit the data of two patients in this study. The points are the observed values of PSA (*left plots*) in ng/ml and hemoglobin (right plots) in g/dl, and the lines show the fits provided by Eq. A. The patient illustrated in the upper left plot provides an example of how the model captured a trend when there were short-lived rises and falls in PSA. Because limited space prevents us from showing similar plots for all of the patients, we illustrate the degree of fit with overall plots of all of the data in Figs. 2 and 3. Fig. 2 shows how the logarithm of fitted values of PSA compared with the logarithm of observed values, and the line on the plot indicates the points at which perfect agreement should occur. Fig. 3 shows an analogous plot for serum hemoglobin. Both plots demonstrate that the model of Eq. A appeared to fit the serial values of these two markers well. The mean difference between observed and fitted log(PSA) was 9.9×10^{-5} (range, −2.8–0.12), and the mean difference between observed and fitted log(hemoglobin) was 1.2×10^{-6} (range, −0.22–0.29). The R^2 values for the linear relationships in Figs. 2 and 3 were respectively 0.98 and 0.92.

Parameter Values Obtained. Table 1 provides the means and ranges of the model's fitted parameters for PSA and hemoglobin. For PSA, the mean value for parameter α of 4.68 implied that the value of PSA at study entry for an average man in our study was ~108 ng/ml The positive values of the means for parameters β and γ imply that, for an average man in our study, PSA steadily increased with time and that the rate of increase in PSA with time also increased with time. In other words, the *rv* for PSA was not constant for most of these men; it increased with time. For hemoglobin, the mean value of parameter α of 2.49 for hemoglobin implied that the average man in our study had a hemoglobin level of 12.1 g/dl on entry onto these studies, and the negative mean values for β and γ implied that hemoglobin dropped with time at ever steeper decreasing rates. The ranges for these parameters also indicate that some men experienced an initial drop in PSA, reflected mostly by negative values of parameter β, and that some also experienced an initial rise in serum hemoglobin, reflected by positive values of parameter β for hemoglobin.

Table 2 Cox proportional hazard model for survival time

Survival time was coded as an interval, beginning with the time of last serum measurement and ending at either the time of death or the time of censoring. The model also included the natural logarithm of the time of the last serum sampling as a covariate, and in this multivariate model, it was also significantly related to survival time ($P = 0.0011$).

Marker	Parameter	Coefficient	SE	*P*
PSA	α	0.112	0.037	0.003
	β	0.746	0.20	0.00016
	γ	2.40	0.77	0.0018
Hemoglobin	α	−1.92	0.47	5.1×10^{-5}
	β	−4.46	1.13	7.6×10^{-5}
	γ	−10.8	2.95	0.00024

Model Parameters and Survival. Table 2 shows the results of a Cox proportional hazard model analysis of the association between the derived parameters and survival time, which was measured using the interval method. To further control for the various times of serum samples, we also included the natural logarithm of the time of last serum measurement as a covariate. The resulting analysis demonstrated that all six exponential model parameters related significantly to subsequent survival time. Higher values of α, β, and γ for PSA implied higher hazard and shorter survival. By contrast, the negative coefficients for hemoglobin implied that higher values of α, β, and γ for hemoglobin coincided with a lower hazard and longer survival. The parameters for hemoglobin contributed significantly to the model, and their use increased the overall model likelihood ratio from 30 to 62.6. Test for a time-dependent trend in the coefficients of the model yielded a global χ^2 value of 11.3 ($P > 0.1$), and the graphs for each coefficient (not shown) demonstrated no trend with time except for parameter γ for hemoglobin. This γ showed a small decline at longer lengths of follow-up, when there were relatively few patients. The goodness of fit test for the final Cox model yielded an overall χ^2 value of 9 ($P > 0.08$). Individual *z* statistics for the deciles of risk ranged from −1.09 to 2.35 (mean, 0.07), and their respective *P*s ranged from 0.025 to 0.4 (mean, 0.3). The single decile of risk with a *P* less than 0.2 had a *z* value of 2.35 ($P = 0.025$), and this occurred at an overall risk score of 0.16. Consequently, we concluded that the Cox model provided a sufficiently good overall fit to the data to give us confidence in the significant way all six of the parameters related to survival.

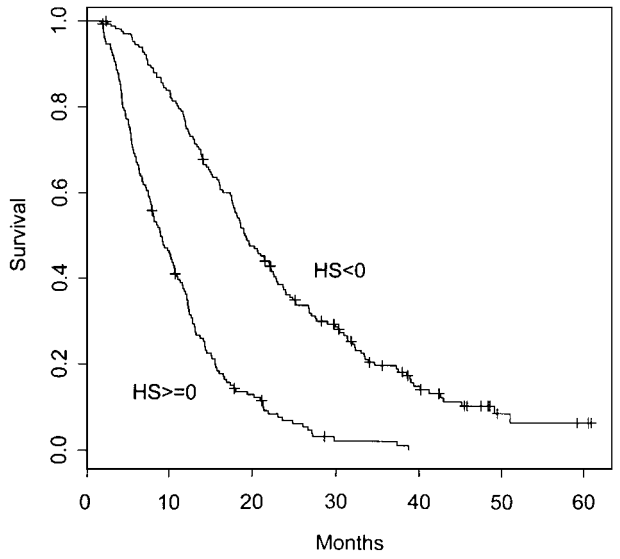

Fig. 4 Kaplan-Meier plots of survival *versus* time of follow-up for those with HS $<$ 0 (*upper curve*) and those with HS $\geq$ 0 (*lower curve*). The HS was based on just the serum-related parameters, and it was calculated from Eq. C.

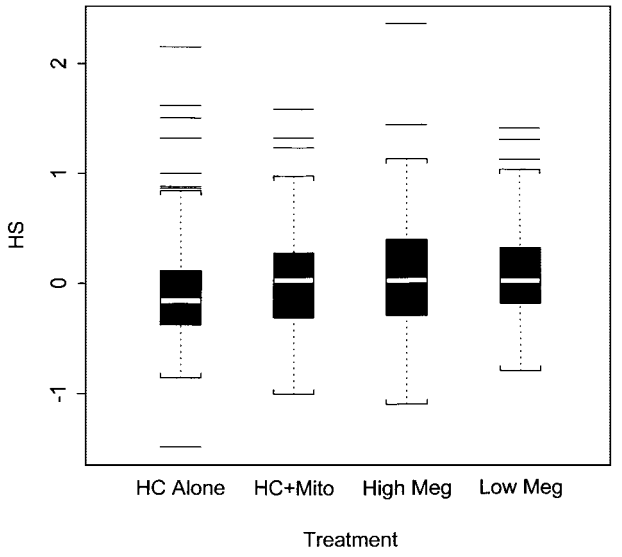

Fig. 5 Boxplots demonstrating the values of HS according to the several treatments used in CALGB 9181 or 9182. *HC Alone*, hydrocortisone alone; *HC+Mito*, the combination of hydrocortisone and mitoxantrone; *High Meg*, megestrol acetate at 640 mg/day; *Low Meg*, megestrol acetate at 160 mg/day. ■, the range of values from the 25th to the 75th percentiles; *white horizontal bars*, median values; *the more distant lines*, the full range.

To illustrate how these parameters may influence survival, we used the coefficients of the Table 2 Cox model to calculate a restricted HS based on just the serum parameters:

$$\begin{aligned} HS = {} & 0.112 * (\alpha 1 - 4.7) + 0.746 * (\beta 1 - 0.05) + 2.40 * (\gamma 1 \\ & - 0.017) - 1.92 * (\alpha 2 - 2.5) - 4.46 * (\beta 2 + 0.007) \quad \text{(C)} \\ & - 10.8 * (\gamma 2 + 0.003) \end{aligned}$$

Here, $\alpha 1$, $\beta 1$, and $\gamma 1$ refer to the model parameters for PSA, and $\alpha 2$, $\beta 2$, and $\gamma 2$ refer to the model parameters for hemoglobin. By subtracting the mean values for each parameter (in the parentheses), we could normalize the HS so that its mean was approximately zero. Thus, for our study patients, HS ranged from -1.49 to 2.37 (mean 0.019). Fig. 4 provides a Kaplan-Meier plot of survival for those with HS $\geq$ 0 (*lower curve*) *versus* those with HS $<$ 0 (*upper curve*). Although this result is not an independent one from the Cox model and in fact weakens the results by dichotomizing HS, it provides a way to see the composite influence of all six parameters on survival in our study population. It also demonstrates that ~50% of our patients achieved a response as reflected by an HS $<$ 0. Finally, Fig. 5 shows how HS compared across the treatment categories used in protocols 9181 and 9182. Here we see that the level of HS among these treatments was very close, and an ANOVA confirmed that there was no difference ($P > 0.3$). Thus, viewed from the standpoint of HS, it is not surprising that there was no difference in survival among these treatments.

DISCUSSION

Our results demonstrate that in men with HRPC, serial values of serum hemoglobin measured during treatment add significant prognostic information to serial values of serum PSA made during the same time. Furthermore, the results suggest that both the level of marker and the first and second derivatives provided additive prognostic information. Although the treatments of these two studies did not significantly improve survival, our analyses demonstrated that, based on their serial values of PSA and hemoglobin, more than 50% of our study patients developed negative values of HS, and our Fig. 5 demonstrates that this majority enjoyed a longer overall survival. Thus, one hypothesis that is raised by our analysis is that HS could provide a measure of response in studies of HRPC. Without additional validating studies with more effective treatments, we cannot prove this hypothesis but offer it as a possible aid to those trying to formulate an effective measure of early response in HRPC.

That serum hemoglobin should be so important is not surprising, because clearly, in HRPC, the level of hemoglobin relates to the tumor burden in the bone marrow, and serum hemoglobin, like body weight, might reflect general cachexia. Practically, however, our results suggest that if we limit serum measurements to PSA during experimental treatments of HRPC, we may identify agents that affect PSA, but not survival. On the other hand if we can identify agents that lower the HS, which combines effects on both PSA and hemoglobin, then such agents should improve survival, because our results suggest that this composite measure is closely related to subsequent survival. Finally, the exponential model we have used seems to fit well the serial values of both PSA and serum hemoglobin during treatment of HRPC, so that it may provide a useful tool to summarize serial serum marker measurements in HRPC.

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