Covariate analysis of viral eradication studies

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SUMMARY
An important issue arising in therapeutic studies of hepatitis C and HIV is the identification of and adjustment for covariates associated with viral eradication and resistance. Analyses of such data are complicated by the fact that eradication is an occult event that is not directly observable, resulting in unique types of censored observations that do not arise in other competing risks settings. This paper proposes a semiparametric regression model to assess the association between multiple covariates and the eradication/resistance processes. The proposed methods are based on a piecewise proportional hazards model that allows parameters to vary between observation times. We illustrate the methods with data from recent hepatitis C clinical trials.

Keywords: Censored observations; Competing risks; Maximum likelihood; Regression.

1. INTRODUCTION
A primary goal of some antiviral trials of hepatitis C and HIV is to suppress and eventually eradicate virus from the host. Such trials have recently been completed for hepatitis C and are ongoing for HIV. In these studies, patients with detectable virus undergo long-term treatment during which their virologic status is monitored using a plasma assay. The assay has a lower limit of detection (LLD), and hence it is not possible to distinguish a viral load level below this limit from complete absence of the virus.

For most patients, an effective antiviral drug will initially drive viral load levels below the assay's LLD. With continued treatment, suppose that the virus in some patients will eventually rebound above the LLD during treatment, an event we refer to as ‘resistance’. For other patients, suppose that undetectable virus is maintained during treatment. The eradication status of such patients cannot be directly assessed due to the assay's LLD. Thus an operational definition of eradication has been developed where patients with undetectable virus have their treatment intentionally withdrawn. If the virus remains undetectable in the absence of treatment for a duration of time referred to as the post-treatment period, then viral ‘eradication’ is assumed to have occurred.

The terms ‘eradication’ and ‘resistance’ are used for simplicity but need not be taken literally. ‘Resistance’, as we have defined it, could be due to the virus developing biological resistance to the drug, in which case viral replication is no longer controlled and viral load levels rebound. In addition, the viral rebound could be due to other factors such as poor drug compliance. Similarly, ‘eradication’ could be the result of complete viral elimination of the virus from the host or to elimination of detectable virus in the plasma though not necessarily in all latent reservoirs. However, it could also result if the host’s immune

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system is capable of indefinitely maintaining viral replication below the LLD in the absence of antiviral treatment.

The goal of this paper is to develop methods for analyzing data from eradication trials to determine whether measured covariates are associated with the risk of development of eradication and resistance. A primary use for such methods is to identify patient characteristics associated with quantities such as the probability of viral eradication within a certain time interval. Such information is useful in making prognoses as well as in determining the optimal duration of therapy. Another use is for comparing the levels of a factor, such as treatment arms in a clinical trial, whilst adjusting for other covariates.

If the times of onset of eradication and resistance were either complete or right-censored, traditional failure time methods for competing risks data could be used to assess the effect of covariates on the cause-specific hazards of resistance and eradication (Kalbfleisch and Prentice, 1980). However, as we will show, the types of observations arising in viral eradication studies result in complex censoring patterns for which standard competing risks methods are not applicable.

Recent scientific advances in the understanding of HIV and hepatitis C suggests that information on viral dynamics, such as the rate of viral clearance, are crucial to the understanding of the disease process (Haase et al., 1996; Neumann et al., 1998). In modeling data from eradication studies, we therefore also consider the patients’ time to first undetectable virus as a fundamental aspect of the formulation. Cheng and Lagakos (2000) developed nonparametric inference methods for the identifiable aspects of the eradication/resistance problem in the one-sample setting and derived the nonparametric maximum likelihood estimators for the subdistribution functions of eradication and resistance in the absence of covariates. In two recently completed hepatitis C eradication trials, covariate analyses were undertaken by regarding patient responses as binary outcomes, with those found to have virus eradicated regarded as ‘successes’ and all others as ‘failures’ (McHutchison et al., 1998; Poynard et al., 1998). However, this approach disregards both viral dynamics and the competing risks structure of the data and may result in an inefficient and biased analysis. As we will show, account of such information in a competing risk model that incorporates viral dynamics can provide a more meaningful interpretation of the results.

An alternative analysis that is also simpler but not in general valid or clinically relevant involves modeling the directly observable quantities of time to first undetectable virus and time to viral rebound. However, the latter depends on the arbitrary experimental conditions, i.e. the chosen probabilities of terminating treatment to assess eradication status in patients with undetectable viral load. We therefore make a clear distinction between suppression during treatment and following treatment. Specifically, viral rebound during treatment is an indication that resistance has occurred, whereas rebound following treatment termination indicates only that neither resistance nor eradication has occurred by the time of treatment termination. Thus, an analysis of the time to viral rebound that does not distinguish between these scenarios would not, in general, be of clinical value. Instead, we focus on the subdistributions of the time to resistance and eradication during treatment.

In Section 2 of this paper, we introduce notation and the statistical model. In Section 3, we discuss the identifiability and estimation of parameters and related issues. In Section 4, we illustrate the methods with data from two recently completed hepatitis C clinical trials.

2. THE MODEL AND LIKELIHOOD FUNCTION

2.1. Notation and observables

Consider a patient with detectable viral load who begins treatment with an antiviral drug at time 0, and whose viral load status is monitored at times $t_1 < t_2 < \cdots < t_M$ using a plasma-based assay. If the drug is initially effective, it will suppress viral load below the assay’s limit of detection. Define $r$ to be the first monitoring time at which the patient has undetectable viral load. With continued treatment,
the patient will eventually either develop resistance or eradication. Let $T$ denote the time from start
of treatment until the development of resistance or eradication, and let $A$ be an indicator of whether $T$
represents the time until eradication ($A = 1$) or resistance ($A = 2$). The joint distribution of $(\tau, T, A)$
can be characterized by the survivorship function $G(u) = Pr(\tau > u)$ and the conditional subdistribution
functions $F_a(t|u) = Pr(T < t, A = a|\tau = u)$, for $a = 1, 2$ and $0 \leq u \leq t < \infty$. Alternatively, the
conditional distribution of $(T, A)$ may be characterized by the cause-specific hazard functions (Prentice
et al., 1978) for eradication and resistance.

Suppose a patient with undetectable virus has treatment intentionally withdrawn and is then monitored
throughout the post-treatment period in order to assess eradication status. The length of the post-treatment
period is determined by the study design and may depend on factors such as the disease under study
and the rate of viral replication. If the virus becomes detectable during the post-treatment period, then
neither resistance nor eradication occurred by the time treatment was discontinued. However, if viral
load remains undetectable throughout the post-treatment period, then the time of onset of eradication is
interval-censored between the time of intervention and the time of termination of treatment. In addition
to the type of incomplete observation resulting from the withdrawal of treatment to assess eradication
status, we also assume that a patient’s outcome can be noninformatively censored due to experimental
conditions (e.g. end-of-study censoring) which can occur prior to or following the initial development
of undetectable viral load. Thus, while we are interested in the distribution of $(\tau, T, A)$, the observed
outcome for a patient is one of five possible types of events, which can be described by $(U, \delta, V, \epsilon)$,
where $U$ is the observed portion of $\tau$, $\delta$ is an indicator of whether $U$ denotes the time of developing
undetectable viral load ($\delta = 1$) or a right-censored observation of $\tau$ ($\delta = 0$), $V$ denotes the observed
portion of $T$, and $\epsilon$ is an indicator of whether the patient did not develop undetectable viral load by time
$V(\epsilon = 0)$, developed resistance at time $V(\epsilon = 1)$, had treatment terminated at time $V$ and virus found
eradicated ($\epsilon = 2$), had treatment terminated at time $V$ and virus not eradicated ($\epsilon = 4$), or was censored
following $\tau$ before developing resistance or having treatment withdrawn ($\epsilon = 3$). In terms of $(\tau, T, A)$,
these five types of observations correspond to the following events:

- **Type 0**: censored at time $t_k$ before developing undetectable virus [$\tau > t_k$];
- **Type I**: developed undetectable virus at time $t_k$ and resistance at time $t_k$, [$\tau = t_k, T = t_k, A = 2$];
- **Type II**: developed undetectable virus at time $t_k$, terminated treatment at
time $t_k$, and virus found eradicated, [$\tau = t_k, t_k \leq T \leq t_k, A = 1$];
- **Type III**: developed undetectable virus at time $t_k$ and censored at time
$t_k$ before developing resistance or terminating treatment,
[$\tau = t_k, [T \geq t_k, A = 2] \cup [T \geq t_k, A = 1]$];
- **Type IV**: developed undetectable virus at time $t_k$, terminated treatment at
time $t_k$, and virus not eradicated, [$\tau = t_k, T > t_k$].

We note that for Type IV outcomes, termination of treatment precludes the future observation of the
time until eradication or resistance as these events are only defined for a patient with uninterrupted treatment.
In Figure 1, we illustrate examples of the Type I–IV outcomes using the observable data.

The overall likelihood function, $L$, based on the five general types of observed outcomes for $N$ independ-
ent patients, factors into one component involving only $G$ and one involving only $F_1$ and $F_2$, i.e.
$L = L_1(G) \times L_2(F_1, F_2)$. Since the data available on $\tau$ are either complete or right-censored, $G(u)$
can be estimated using standard methods for failure time data. We therefore focus on the estimation of $F_2(t|u)$
and $F_2(t|u)$ based on $L_2$. 

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Fig. 1. Examples of observed data for Type I-IV outcomes are given where \( \oplus (\ominus) \) denotes a measurement of undetectable (detectable) virus during treatment, and \(- (+)\) denotes the patient stopped treatment and virus eradicated (not eradicated). The above data correspond to the following censoring patterns for \( T \), time to eradication \((A = 1)\) or resistance \((A = 2)\), and \( A \): (a) \( T = t_3, A = 2 \), (b) \( t_1 \leq T \leq t_3, A = 1 \), (c) \( \{ T > t_3, A = 2 \} \cup \{ T \geq t_1, A = 1 \} \), (d) \( T > t_3 \).

2.2. The model

Let \( \pi_j \) denote the probability that a patient with undetectable viral load at time \( t_j \) has treatment terminated to assess eradication status. For the one-sample problem, Cheng and Lagakos (2000) show that \( F_2(t|u) \) is identifiable at \( t = t_1, t_2, \ldots, t_M \) and \( F_1(t|u) \) is identifiable at those \( t_j \) for which \( \pi_j > 0 \). Thus, for the one-sample problem, it is convenient to model the joint distribution of \((T, A)\) in terms of subdistribution functions. However, the possible effects of covariates on the distribution of \((T, A)\) are more naturally modeled in terms of the cause-specific hazard functions (Prentice et al., 1978) for eradication and resistance, say \( \lambda^E(t) \) and \( \lambda^R(t) \), where dependence on \( \tau \) is suppressed hereafter for notational simplicity.

Define \( \delta_j = 1[\pi_j > 0] \) and denote the treatment stopping interval at \( t_j \) as \( g(j) = 1 + \sum_{k=1}^{j-1} \delta_k \) for \( j = 1, \ldots, M \). Also define \( M_1 = g(M) \). Suppose that

\[
\lambda^E_0(t) = \theta_{g(j)} \lambda(t)
\]

and

\[
\lambda^R_0(t) = (1 - \theta_{g(j)}) \lambda(t),
\]

for \( t_{j-1} < t \leq t_j \), where \( \lambda(t) \) is arbitrary and \( 0 \leq \theta_{g(j)} \leq 1 \). This model assumes the \( \lambda^E_0(t) \) and \( \lambda^R_0(t) \) are proportional within the treatment stopping intervals. Define \( \Delta_j = \Lambda(t_j) - \Lambda(t_{j-1}) \) where \( \Lambda(t) = \int_0^t \lambda(u) du \). It can be shown that the subdistribution functions for eradication and resistance can
be expressed in terms of the cause-specific hazard functions as follows:

\[ F_1(t_k) = \sum_{h=1}^{k} (1 - \theta_{g(h)}) \times [1 - \exp(-\Delta_h)] \times \prod_{i=0}^{h-1} \exp(-\Delta_i) \]

\[ F_2(t_j) = \sum_{h=1}^{j} \theta_{g(h)} \times [1 - \exp(-\Delta_h)] \times \prod_{i=0}^{h-1} \exp(-\Delta_i) \]

for \( j = 1, \ldots, M \) and \( k \) such that \( \tau_k > 0 \). Note that the \((M_1 + M)\) terms on the left-hand side define the identifiable aspects of the joint distribution of \((T, A)\) for the one-sample problem. Since \((\Delta_1, \ldots, \Delta_M, \theta_1, \ldots, \theta_{M_1})\) is a 1–1 function of the \((M_1 + M)\) identifiable values of \( F_2(\cdot) \) and \( F_1(\cdot) \), it follows that the assumption of proportionality of \( \lambda^R(t) \) and \( \lambda^E(t) \) within intervals is not restrictive. As will be illustrated in Section 5, the estimated subdistributions from this model, in the case of a single, binary covariate, are equivalent to the nonparametric estimators derived by Cheng and Lagakos (2000) for the one-sample problem.

Let \( z \) denote the \((1 \times p)\) covariate vector available for all subjects, and let \( \lambda^R(t, z) \) and \( \lambda^E(t, z) \) denote the cause-specific hazard functions for resistance and eradication, respectively. We assume the following piecewise proportional hazards models for \( \lambda^R(t, z) \) and \( \lambda^E(t, z) \):

\[ \lambda^R(t, z) = \lambda^R_0(t) \exp(z\beta^R_j) \]

\[ \lambda^E(t, z) = \lambda^E_0(t) \exp(z\gamma^E_{g(j)}) \]

for \( t_{j-1} < t \leq t_j \), where \( \beta^R_j \) and \( \gamma^E_{g(j)} \) are the corresponding \((p \times 1)\) coefficient vectors for resistance and eradication, respectively, and \( \lambda^R_0(t) \) and \( \lambda^E_0(t) \) are the underlying cause-specific hazard functions for resistance and eradication. This model allows the regression coefficients, \( \beta^R_j \) and \( \gamma^E_{g(j)} \), to vary between the timepoints at which resistance and eradication are observed. Thus \( \exp(\{z_1 - z_2\}/\gamma_{g(j)}^E) \) denotes the cause-specific hazard ratio of resistance, \( \lambda^R(t, z_1)/\lambda^R(t, z_2) \), for \( t \in (t_{j-1}, t_j] \), where \( z_1 \) and \( z_2 \) denote two specific values of the covariate vector \( z \). The regression coefficient \( \gamma^E_{g(j)} \) has a similar interpretation.

### 2.3. The likelihood function

Using the model given in section 2.2, the likelihood contributions of the Type I–IV events for a patient with covariate vector \( z \) can be expressed as

**Type I:** \((c^R_j/c^E_j) \times [1 - \exp(-\Delta_j c^E_j)] \times \prod_{h=0}^{j-1} \exp(-\Delta_h c^E_h)\)

**Type II:** \(\sum_{h=1}^{j} (c^E_{g(h)}/c^E_j) \times [1 - \exp(-\Delta_h c^E_h)] \times \prod_{i=0}^{h-1} \exp(-\Delta_i c^E_i)\)

**Type III:** \(1 - \sum_{h=0}^{j-1} [(c^R_h/c^E_h) \times [1 - \exp(-\Delta_h c^E_h)] \times \prod_{i=0}^{h-1} \exp(-\Delta_i c^E_i)]\)

**Type IV:** \(\prod_{h=1}^{j} \exp(-\Delta_h c^E_h)\)

where \(c^E_{g(j)} = (1 - \theta_{g(j)}) \exp(z\gamma^E_{g(j)}) \), \(c^R_j = \theta_{g(j)} \exp(z\beta^R_j)\), and \(c^E_j = c^R_{g(j)} + c^E_{g(j)}\). The likelihood function, \(L_2\), is a product of the above types of contributions. If \( \tau = t_0 \), then \( \Delta_j = 0 \) as neither eradication nor resistance can occur until after the patient has developed undetectable virus. Thus for two groups with \( \tau = t_1 \), our goal is to estimate the \(2(M_1 + M)\) quantities \( \beta^R_j, \gamma^E_{g(j)} \), and \( \Delta_j \), for \( j = s, \ldots, M \).

### 3. Identifiability and estimation of parameters

For each \( \tau \), the identifiable aspects of the model are \( \beta^R_j, \gamma^E_{g(j)}, \Delta_j \), and \( \theta_{g(j)} \) for \( j = 1, \ldots, M \). In some applications, such as in the example described in the following section, a patient cannot, by definition,
develop resistance at the same visit time as they first develop undetectable virus, in which case $\beta_1$ is undefined. Similarly, $\theta_{g(j)}$ will be estimable for each $g(j) = 1, \ldots, M_1$ if there is at least one observed event of either eradication or resistance at each $t_j$ for which $\pi_j = 1$. Since $\theta_{g(j)}$ is constant within a treatment stopping interval, the cumulative hazard of eradication will be estimable at all times at which the cumulative hazard of resistance is identifiable using the relationship $\lambda_o^E(t) = \frac{(1-\theta_{g(j)})}{\theta_{g(j)}} \lambda_o^R(t)$. It follows that the overall baseline hazard, $\Lambda(t)$, is identifiable at each $t_1, \ldots, t_M$, and that $\Delta_j$ is estimable for all $j = 1, \ldots, M$

Suppose $M$ is fixed and $N \to \infty$. Since the dimension of $(\gamma, \beta, \theta, \Delta)$ is fixed and finite-dimensional, standard large-sample likelihood results apply under the usual regularity conditions (Cox and Hinkley, 1974). The maximum likelihood estimators (MLEs) of $(\gamma, \beta, \theta, \Delta)$ for each $\tau$ are not obtainable in closed form, but can be obtained by maximizing $L_2$ using standard numerical methods. The covariance matrix of $(\hat{\gamma}, \hat{\beta}, \hat{\theta}, \hat{\Delta})$ is similarly estimated via the inverse of the observed information matrix corresponding to $L_2$. Since patients are assumed to be independent observations and the data from different $\tau$ groups are modeled separately, the estimated parameters corresponding to different $\tau$ groups are asymptotically independent. Estimated variances for functions of the MLEs can be obtained from the estimated covariance matrix of the MLEs using the delta method. For example, an estimated standard error for $\exp(\hat{\gamma})$ is given by $\hat{v} \cdot \exp(\hat{\gamma})$, where $\hat{v}$ is the estimated standard error of $\hat{\gamma}$.

4. Model reduction

The model proposed in Section 2.2 allows the effects of a covariate to vary with time. However, it may be of interest to know whether a more parsimonious model fits the data. If the cause-specific hazard ratio corresponding to the levels of a covariate is constant in time, then the model may be reduced to a simpler form. Thus it may be of interest to test the following null hypotheses:

\begin{itemize}
  \item[(1)] $H_R : \beta_1 = \cdots = \beta_M$
  \item[(2)] $H_E : \gamma_1 = \cdots = \gamma_{M_1}$
  \item[(3)] $H_O : H_R \cap H_E$
\end{itemize}

Under $H_O$ ($H_R$), the covariate $z$ exerts a proportional hazards effect on resistance (eradication), whereas $H_O$ denotes a full proportional hazards model for the eradication/resistance processes. Standard likelihood ratio tests may be used to test $H_R$ in order to compare the above model with the full model proposed in Section 2.2. The above model is easily modified to test whether only specific regression coefficients are constant over time.

5. Example

We illustrate the proposed methods with data from two recently completed and similarly designed clinical trials comparing interferon plus placebo (IP) to interferon plus ribavirin (IR) in patients with chronic hepatitis C virus (HCV). The primary endpoint of the trials was a sustained virologic response, defined as the absence of detectable serum HCV RNA 24 weeks following the termination of treatment. In both trials, the objective was to assess the effects of treatment on the probability of sustained virologic suppression of hepatitis C, and to identify factors associated with sustained response. A total of 1744 patients were randomly assigned to receive IR for 24 weeks (505 patients), IR for 48 weeks (505) patients, IP for 24 weeks (235 patients), or IP for 48 weeks (499 patients). Note in the study by Poynard et al. (1998), no patients were randomized to IP for 24 weeks, thus resulting in an imbalance of the treatment groups in the combined study. Viral load was monitored at 4, 12, 24, 36, and 48 weeks during the course of treatment. For a complete description of these studies, see McHutchison et al. (1998) and Poynard et
al. (1998). Hereafter we use ‘eradication’ to refer to a sustained response and ‘resistance’ to refer to the re-emergence of detectable virus during the treatment period.

In the analyses by McHutchison et al. (1998) and Poynard et al. (1998), eradication was treated as a binary outcome where patients who were not observed to achieve viral eradication (Types 0, 1, III, and IV) were classified as failures. In addition, patients given the same treatment but for different durations were treated as distinct groups, and thus the trials were analysed and interpreted as a 4-arm trial.

We reanalysed these data using the formulation and methods described in previous sections. In the notation of this paper, \( M = 5 \), \( M_1 = 2 \), \( t = (4, 12, 24, 36, 48) \), \( \pi = (0, 0, 3/7, 0, 1) \), \( g(j) = 1, 1, 1, 2, 2 \) for \( j = 1, 2, \ldots, 5 \). We view the combined trial as having two arms (IR versus IP), with treatment durations in each arm of 24 or 48 weeks, as determined by the \( \pi_j \). Because of the definition of resistance used in this example, the resistance subdistribution function is only estimated at monitoring times after \( \tau \) as detectable viral load cannot be measured at the same time that first undetectable virus is measured. For example, when \( \tau = 4 \) weeks, the resistance subdistribution function is estimable at weeks 12, 24, 36, and 48, whereas the eradication subdistribution function is estimable at weeks 24 and 48, the possible times of treatment termination. Of the 1744 patients, 49\% developed resistance (Type I event), 26\% achieved viral eradication (Type II event), and 14.3\% developed undetectable viral load, but did not achieve eradication (Type IV event). The remaining 2.8\% of the subjects had incomplete histories (Type III event), possibly from censoring.

A univariate analysis of the time to first undetectable virus was performed using the Kaplan–Meier estimator. Patients in the IR group achieved initial undetectable virus at a significantly higher rate than those in the IP group (\( p < 0.001 \), logrank test). An estimated 22\%, 29\%, 9\%, 1\%, and 1\% of the patients in the IR group developed first undetectable virus at weeks 4, 12, 24, 36, and 48 weeks, respectively, compared with 12\%, 16\%, 9\%, 2\%, and 1\% in the IP group. To compare the risks of eradication and resistance for the two treatments, conditional on time to first undetectable virus, the models of Section 2.2 were fit to the data.

5.1. Unadjusted treatment comparisons

Suppose that \( z \) is a scalar covariate denoting treatment group (\( z = 0 \) for IP and \( z = 1 \) for IR). For each \( \tau = 4, 12, \) and 24 weeks, we first fit the following model:

\[
\lambda^R(t, z) = \begin{cases} 
\theta_1 \lambda(t) \exp(\beta_2 z), & 4 < t \leq 12 \\
\theta_2 \lambda(t) \exp(\beta_3 z), & 12 < t \leq 24 \\
\theta_2 \lambda(t) \exp(\beta_4 z), & 24 < t \leq 36 \\
\theta_2 \lambda(t) \exp(\beta_5 z), & 36 < t \leq 48 
\end{cases}
\]

\[
\lambda^E(t, z) = \begin{cases} 
(1 - \theta_1) \lambda(t) \exp(\gamma_1 z), & 4 < t \leq 24 \\
(1 - \theta_2) \lambda(t) \exp(\gamma_2 z), & 24 < t \leq 48.
\end{cases}
\]

Thus, for \( \tau = 4 \) weeks, there are 12 parameters to be estimated (\( \beta_2, \beta_3, \beta_4, \beta_5, \gamma_1, \gamma_2, \theta_1, \theta_2, \Delta_2, \Delta_3, \Delta_4, \Delta_5 \)). The MLEs for Model (1) are presented in Table 1. For example, for patients who developed undetectable virus by week 4, the incremental change in the cumulative hazard, \( \Delta \), is estimated as 0.20, 0.73, 0.56, and 1.51 at weeks 12, 24, 36, and 48, respectively. The estimated proportion of the overall hazard function attributable to resistance, \( \hat{\Delta} \), is 19\% from week 4 to 24 and is 18\% from week 24 to 48. The treatment hazard ratio of resistance, \( \exp(\hat{\beta}) \), is estimated as 0.93, 0.27, 0.0, and 0.80 for weeks 12, 24, 36, and 48, respectively. The estimate of \( \exp(\hat{\beta}) \) at week 36 is zero as no resistance events occurred in the IR group at that time. The estimated treatment hazard ratio of eradication, \( \exp(\hat{\gamma}) \), and corresponding estimated 95\% confidence intervals, is 3.29 (1.68, 6.44) from week 4 to 24 and 0.44 (0.07, 2.79) from
eradication is possible at week 24, while resistance is not. That is, some patients with τ terminated treatment at week 24 and viral eradication was observed. However, patients with τ week 24 to 48. For patients with τ = 336 D. M. CHENG AND S. W. LAGAKOS

were patients in the IR group with weeks have undetectable virus and, therefore, could not develop resistance at week 24. Also, since there interpretable subdistributions of eradication and resistance using the following equations:
equivalence of this saturated model to the one-sample approach. The data indicate that for every IP group. Also, for both treatment groups, the probability of eradication by week 24 or 48 decreases markedly with τ, the time at which viral load first becomes undetectable. Formulation of the problem

<table>
<thead>
<tr>
<th>Time (weeks)</th>
<th>12</th>
<th>24</th>
<th>36</th>
<th>48</th>
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<tbody>
<tr>
<td>τ = 4 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ</td>
<td>0.20 (0.11)</td>
<td>0.73 (0.25)</td>
<td>0.56 (0.39)</td>
<td>1.51 (0.67)</td>
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<tr>
<td>exp(β)</td>
<td>0.93 (0.61)</td>
<td>0.27 (0.24)</td>
<td>0.00</td>
<td>0.80 (0.98)</td>
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<td>τ = 12 weeks</td>
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<tr>
<td>Δ</td>
<td>–</td>
<td>0.31 (0.08)</td>
<td>0.57 (0.15)</td>
<td>0.26 (0.14)</td>
</tr>
<tr>
<td>exp(β)</td>
<td>–</td>
<td>0.50 (0.16)</td>
<td>0.74 (0.37)</td>
<td>2.73 (1.99)</td>
</tr>
<tr>
<td>τ = 24 weeks</td>
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<td></td>
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<tr>
<td>Δ</td>
<td>–</td>
<td>–</td>
<td>0.44 (0.13)</td>
<td>0.33 (0.14)</td>
</tr>
<tr>
<td>exp(β)</td>
<td>–</td>
<td>–</td>
<td>0.73 (0.38)</td>
<td>0.82 (0.62)</td>
</tr>
<tr>
<td>τ = 4 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Δ</td>
<td>–</td>
<td>–</td>
<td>0.19 (0.16)</td>
<td>–</td>
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<tr>
<td>exp(β)</td>
<td>–</td>
<td>–</td>
<td>3.29 (1.13)</td>
<td>–</td>
</tr>
<tr>
<td>τ = 24 weeks</td>
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<tr>
<td>Δ</td>
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<td>–</td>
<td>0.65 (0.29)</td>
<td>–</td>
</tr>
<tr>
<td>exp(β)</td>
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<td>–</td>
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<td>–</td>
</tr>
<tr>
<td>τ = 24 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ</td>
<td>–</td>
<td>–</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>exp(β)</td>
<td>–</td>
<td>–</td>
<td>∞</td>
<td>–</td>
</tr>
</tbody>
</table>

The results are given in Table 2 and coincide with the estimates in Cheng and Lagakos (2000) using nonparametric techniques for one-sample estimation, illustrating the equivalence of this saturated model to the one-sample approach. The data indicate that for every τ, patients receiving the IR therapy achieve viral eradication sooner and at a higher rate than those in the IP group. Also, for both treatment groups, the probability of eradication by week 24 or 48 decreases markedly with τ, the time at which viral load first becomes undetectable. Formulation of the problem

\begin{align*}
F_1(t_k) &= \frac{k}{\sum_{h=1}^{k} (c_{E(h)}^E / c_{ER}^E) \times \left[ 1 - \exp(-\Delta_b c_{ER}^E) \right] \times \prod_{i=b}^{k-1} \exp(-\Delta_i c_{ER}^E)}
\end{align*}

\begin{align*}
F_2(t_j) &= \frac{j}{\sum_{h=1}^{j} (c_{E(h)}^E / c_{ER}^E) \times \left[ 1 - \exp(-\Delta_b c_{ER}^E) \right] \times \prod_{i=b}^{j-1} \exp(-\Delta_i c_{ER}^E)}
\end{align*}

for k = 3, 5 and j = 2, \ldots, 5. The results are given in Table 2 and coincide with the estimates in Cheng and Lagakos (2000) using nonparametric techniques for one-sample estimation, illustrating the equivalence of this saturated model to the one-sample approach. The data indicate that for every τ, patients receiving the IR therapy achieve viral eradication sooner and at a higher rate than those in the IP group. Also, for both treatment groups, the probability of eradication by week 24 or 48 decreases markedly with τ, the time at which viral load first becomes undetectable. Formulation of the problem
in terms of the random variables $\tau$, $T$, and $A$ is useful in determining the optimal duration of therapy for a patient. The estimates for $Pr(24 < T \leq 48, A = 1|\tau)$ can be used to assess the effectiveness of 24 versus 48 weeks as this quantity represents the additional probability of eradication gained from extending treatment 24 weeks. For example, patients in the IR group increase their probability for viral eradication by 0.04, 0.25, and 0.30 given the time to first undetectable virus is at week 4, 12, and 24 weeks, respectively. Thus, with respect to eradication, an additional 24 weeks of IR treatment was more beneficial to patients who first developed undetectable virus after week 4, whereas there may be little to gain by extending IR treatment beyond week 24 in patients whose viral load became undetectable by week 4. With respect to resistance, the results suggest that the probability of developing resistance is greater for the IP group and that, for both the IR and IP groups, the probability of developing resistance increases with the time to first undetectable virus.

The results of Model (1) are presented conditional on $\tau$ as such results are helpful in elucidating the relationship between viral dynamics and the eradication/resistance processes. However, the primary analysis may be unconditional comparisons of treatment effects. In such cases, the unconditional results may be obtained from the conditional estimates. For example, using the MLEs in Table 1, we estimated the unconditional subdistribution function of eradication for the IR and IP treatment groups. The results indicate that the unconditional probability of eradication by week 48 (standard error) for the IR and IP groups is 0.46 (0.03) and 0.16 (0.02), respectively.

We next considered the special case of Model (1) in which the treatment effect on the cause-specific hazard for resistance is time independent (i.e. a PH model) but the treatment effect on eradication may vary with time, i.e.

$$
\lambda^E(t, z) = \begin{cases} 
\theta_1 \lambda(t) \exp(\beta z), & 4 < t \leq 24 \\
\theta_2 \lambda(t) \exp(\beta z), & 24 < t \leq 48
\end{cases}
$$

$$
\lambda^R(t, z) = \begin{cases} 
(1 - \theta_1) \lambda(t) \exp(\gamma_1 z), & 4 < t \leq 24 \\
(1 - \theta_2) \lambda(t) \exp(\gamma_2 z), & 24 < t \leq 48
\end{cases}
$$

The resulting hazard ratios of eradication and resistance are given in Table 3. A likelihood ratio test of $H_{CSR}$: $\beta_2 = \cdots = \beta_5$ is not significant for any of the $\tau$ groups ($p = 0.55$, $p = 0.09$, $p = 0.87$, for $\tau = 4, 12, \text{and} 24$ weeks, respectively), suggesting that this more parsimonious model does not provide...
Table 3. Estimated hazard ratios of treatment for 
Model (2) (95% CI)

<table>
<thead>
<tr>
<th>τ</th>
<th>Resistance</th>
<th>Eradication</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>t ≤ 48</td>
<td>t ≤ 24</td>
</tr>
<tr>
<td>4</td>
<td>0.59 (0.24, 1.46)</td>
<td>3.56 (1.80, 6.90)</td>
</tr>
<tr>
<td>12</td>
<td>0.68 (0.41, 1.13)</td>
<td>5.93 (1.88, 18.9)</td>
</tr>
<tr>
<td>24</td>
<td>0.76 (0.34, 1.68)</td>
<td>∞</td>
</tr>
</tbody>
</table>

an unreasonable fit to the data. The results of Model (2) are not greatly different from those obtained from Model (1). For example, for those patients on IR therapy who first developed undetectable virus at week 4, Model (2) estimates the probability of eradication by week 24 as 0.89 and the probability of eradication between weeks 24 and 48 as 0.03. For the IP group, these probabilities are estimated as 0.47 and 0.30. Both models indicate that the treatment effect on eradication is highly significant within the first 24 weeks of therapy (Models (1) and (2); p < 0.001) but not significant between weeks 24 and 48 Models (1): p = 0.38, Model (2): p = 0.34).

5.2. Assessing the effects of several explanatory variables

In addition to treatment group, we next consider the effects of HCV genotype, age, and weight on the cause-specific hazards of resistance and eradication. Each of the latter three covariates has been shown to affect treatment responses in hepatitis C (Pawlotsky et al., 1996). We fit the following model, which assumes that the covariate effect on the cause-specific hazard for resistance is a PH model, and the covariate effect on eradication may vary with time:

\[
\lambda^R(t, z) = \begin{cases}
\theta_1 \lambda(t) \exp(\beta_1 z_1 + \beta_2 z_2 + \beta_3 z_3 + \beta_4 z_4), & 4 < t \leq 24 \\
\theta_2 \lambda(t) \exp(\beta_1 z_1 + \beta_2 z_2 + \beta_3 z_3 + \beta_4 z_4), & 24 < t \leq 48
\end{cases}
\]

\[
\lambda^E(t, z) = \begin{cases}
(1 - \theta_1) \lambda(t) \exp(\gamma_1 z_1 + \gamma_2 z_2 + \gamma_3 z_3 + \gamma_4 z_4), & 4 < t \leq 24 \\
(1 - \theta_2) \lambda(t) \exp(\gamma_1 z_1 + \gamma_2 z_2 + \gamma_3 z_3 + \gamma_4 z_4), & 24 < t \leq 48.
\end{cases}
\]  

(3)

Here \( z_1 \) is the treatment indicator defined previously, \( z_2 \) is defined as 1 for patients with HCV genotype 1 and 0 otherwise, and \( z_3 \) and \( z_4 \) denote log of age and log of bodyweight at the start of treatment, respectively. The estimated hazard ratios corresponding to Model (3) are given in Table 4. We first note that the estimated treatment effects are generally similar to those obtained in the preceding model which did not account for covariates. Both models indicate that patients in the IR group have a significantly higher hazard of viral eradication by week 24 compared with patients in the IP group and that the treatment effect increases with the time to first undetectable virus.

Of the remaining three covariates, age is not significantly associated with the risk of either resistance or eradication in patients who develop undetectable viral load by week 12. However, patients with HCV genotype 1 have a significantly lower risk of viral eradication by week 24 than those with other genotypes, regardless of the time it takes to develop undetectable viral load. The effects of bodyweight on the risk of resistance and eradication is less clear. Bodyweight appears to be positively associated with the risk of resistance among patients who achieve first undetectable viral load at week 12. However, it is also positively associated with the risk of viral eradication by week 24 for those who develop first undetectable virus by week 4.

Using Model (3), the hypothesis that treatment is not associated with either eradication or resistance is
Table 4. Estimated hazard ratios from Model (3) (95% CI)

<table>
<thead>
<tr>
<th>τ</th>
<th>Covariate</th>
<th>Resistance $t \leq 48$</th>
<th>Resistance $t \leq 24$</th>
<th>Eradication $24 &lt; t \leq 48$</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Treatment</td>
<td>0.63 (0.25, 1.55)</td>
<td>4.26 (2.14, 8.48)</td>
<td>0.16 (0.01, 4.34)</td>
</tr>
<tr>
<td></td>
<td>Genotype</td>
<td>2.10 (0.88, 4.98)</td>
<td>0.61 (0.38, 0.98)</td>
<td>3.06 (0.45, 20.9)</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>0.89 (0.15, 5.27)</td>
<td>0.79 (0.32, 1.97)</td>
<td>0.14 (0.002, 12.0)</td>
</tr>
<tr>
<td></td>
<td>Weight</td>
<td>1.12 (0.12, 10.5)</td>
<td>4.17 (1.15, 15.1)</td>
<td>0.24 (0.001, 60.3)</td>
</tr>
<tr>
<td>12</td>
<td>Treatment</td>
<td>0.67 (0.40, 1.12)</td>
<td>7.59 (2.42, 23.8)</td>
<td>1.90 (0.93, 3.86)</td>
</tr>
<tr>
<td></td>
<td>Genotype</td>
<td>0.99 (0.59, 1.66)</td>
<td>0.41 (0.25, 0.67)</td>
<td>0.82 (0.40, 1.66)</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>0.77 (0.22, 2.71)</td>
<td>0.60 (0.18, 2.01)</td>
<td>1.54 (0.32, 7.34)</td>
</tr>
<tr>
<td></td>
<td>Weight</td>
<td>4.66 (1.47, 14.96)</td>
<td>1.05 (0.36, 3.09)</td>
<td>0.20 (0.04, 1.14)</td>
</tr>
<tr>
<td>24</td>
<td>Treatment</td>
<td>0.58 (0.24, 1.41)</td>
<td>$\infty$</td>
<td>2.43 (0.84, 7.1)</td>
</tr>
<tr>
<td></td>
<td>Genotype</td>
<td>2.69 (0.87, 8.31)</td>
<td>0.10 (0.03, 0.37)</td>
<td>1.77 (0.49, 6.41)</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>1.79 (0.20, 16.0)</td>
<td>0.07 (0.001, 3.78)</td>
<td>0.06 (0.004, 0.80)</td>
</tr>
<tr>
<td></td>
<td>Weight</td>
<td>6.05 (0.70, 52.7)</td>
<td>10.7 (0.36, 314)</td>
<td>3.16 (0.22, 44.6)</td>
</tr>
</tbody>
</table>

given by $H_0 : \beta_1 = \gamma_{11} = \gamma_{21} = 0$. The results for $\tau = 4$ and 12 weeks indicate that the overall treatment difference is highly significant in both $\tau$ groups ($p < 0.001$, in both cases).

6. DISCUSSION

A fundamental feature of the proposed methods is their use of information on viral dynamics. This reflects the central role of viral load in the determination of treatment and treatment duration in persons with HIV, and the growing opinion that therapeutic strategies in hepatitis C should target sustained virologic suppression.

Because the subdistribution functions of resistance and eradication are identifiable only at times where viral load is monitored, it is more natural to introduce covariate effects via the cause-specific hazard functions than on the subdistribution functions. In this paper, we used multiplicative intensity models for the cause-specific hazards of resistance and eradication. An alternative approach would be to use an accelerated failure time model to incorporate covariate effects (Kalbfleisch and Prentice, 1980).

In most applications of the proposed methods, we expect that analyses will focus on the effects of treatments and covariates on the risk of eradication, as viral eradication is the therapeutic goal in some chronic diseases such as hepatitis C. However, in developing optimal treatment strategies, it is often critical to identify factors associated with the development of resistance. This may be very important in studies of HIV, in which case there may be equal emphasis on the association between covariates and the cause-specific hazards of resistance and eradication.

Although we assume that the outcome of ‘resistance’ is a terminal event, a dataset might include some observations where the virus has been suppressed below the LLD, re-emerges above the LLD at a later time during treatment, and then returns to undetectable levels throughout the remainder of the treatment and post-treatment periods. Such an occurrence may be the result of factors such as measurement error and/or natural variability in virus levels. To avoid such inconsistencies in the data, the operational definition of ‘resistance’ could be redefined, for example, as the re-emergence of detectable virus in consecutive measurements during the treatment period.
There are many aspects of the design, conduct, and interpretation of viral eradication studies that require further research. One aspect with respect to study design is the determination of an appropriate length for the post-treatment period. This is an important concern because the definition of ‘eradication’ depends on the length of the post-treatment period. If the length of the post-treatment period is not sufficient, then the outcome of ‘eradication’ may not have a clinically meaningful interpretation. However, if the post-treatment period is too long, there is potential harm in terminating treatment in a patient who has not achieved viral eradication.

In studies of HIV, where treatments may be given for several years and where the possibility of complete viral elimination is a controversial issue, the development of adaptive designs in which \( t_j \) and \( \pi_j \) depend on the history of the process up until time \( t_{j-1} \) are needed. Also, the consequences of terminating treatment in a patient who has not achieved viral eradication are unknown. Some researchers have speculated that it may be difficult to re-suppress viral load to undetectable levels once virus has rebounded as a result of treatment termination. Thus during the early stages of a trial, if eradication rates appear to be low, it may be desirable to either delay subsequent times of treatment termination or reduce the proportion of patients who terminate treatment. Specific adaptive designs that reflect such strategies and their properties require further investigation. Research is also needed for situations where patients may have different monitoring times, \( t_j \), so that \( M \to \infty \) as \( N \to \infty \). In such cases, large sample properties of MLEs do not rely on standard likelihood methods.

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REFERENCES


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