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A test of homogeneity for age-dependent branching processes with immigration

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Abstract: We propose a novel procedure to test whether the immigration process of a discretely observed age-dependent branching process with immigration is time-homogeneous. The construction of the test is motivated by the behavior of the coefficient of variation of the population size. When immigration is time-homogeneous, we find that this coefficient converges to a constant, whereas when immigration is time-inhomogeneous it is time-dependent, at least transiently. Thus, we test the assumption that the immigration process is time-homogeneous by verifying that the sample coefficient of variation does not vary significantly over time. The test is simple to run and does not require specification or fitting any branching process to the data. Its implementation is identical whether the process is sub-, super-, or critical. Simulations and an application to real data on the progression of leukemia are presented to illustrate the approach.

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1. Introduction

Age-dependent branching processes define a class of continuous-time stochastic processes that is increasingly popular in the biological sciences. They offer flexible, yet tractable, stochastic models that describe population dynamics at the individual level, and allow the lifespan to follow arbitrary distributions (e.g., [1, 2, 3, 4, 5, 6, 7]). They can be extended in a number of useful ways to adapt to specific situations. For example, an immigration component may be included to describe an influx of immigrants into the population of interest.

Sevastyanov (1957) was the first to study branching processes with immigration [8]. He investigated the Markov case. Extensions to age-dependent processes were subsequently considered by Jagers and other authors in the single-type [9, 10, 11, 12, 13, 14, 15, 16, 17, 18] and the multi-type case [19, 20]. Recently, this class of processes has been proposed to model the dynamics of cell populations *in vivo* under the assumption that immigration obeys a (possibly time-inhomogeneous) Poisson process [17, 18, 20, 21].

Statistical inference for the Bienaymé-Galton-Watson process with immigration has been extensively studied and is discussed in Guttorp's monograph [22]. In contrast, statistical inference for age-dependent branching processes with immigration has received little, if any, attention to date. In particular, no methods have been developed to determine whether the rate of the immigration process should be taken as constant or allowed to vary with time. We see two reasons for developing a procedure that addresses this question: firstly, it is not always known in practice whether the influx of cells in the population of interest changes over time such that the procedure could lead to valuable biological insights; secondly, from a statistical standpoint, it would help to decide whether the data can support a model with time-inhomogeneous immigration, and thus could prevent over-parameterization issues. The question of how to specify the shape of the immigration rate is also important, but we do not address it here.

The goal of this paper is to propose a test to determine whether the immigration process of an age-dependent branching process with immigration is time-homogeneous by only observing the population size. The proposed test applies when several independent populations are observed at discrete time points; such experimental designs are commonly used in biology. We first investigate the asymptotic behavior of the coefficient of variation of the population size under various immigration rates and when the branching process is sub-, super-, and critical. We find that the coefficient of variation converges over time to a strictly positive constant when the immigration process is time-homogeneous. In contrast, when the immigration process is time-inhomogeneous, we find that the coefficient of variation is either time-dependent, possibly after applying a suitable transformation, or transits to a different constant. Thus, we construct a test which verifies if the empirical coefficient of variation changes significantly over time, which is accomplished by techniques of linear regression.

An attractive feature of the test is that it is simple to implement. In particular, it does not require any branching process to be fitted to the data, and it does not impose either that the distribution of the lifespan and the shape of the immigration rate, should it be time-dependent, be formulated. Its implementation is identical whether the process is sub-, super-, or critical. This simplicity is a consequence of the fact that the test is solely constructed from the asymptotic behavior of the process. Statistical methods for branching processes that rely on their asymptotic behavior have been successfully used in the past [22, 23, 24, 25, 26, 27], and the proposed test is built in the same vein. Asymptotic procedures for testing the homogeneity of coefficients of variation across samples have also been proposed [28, 29] (see also [30] for a derivation of the asymptotic distribution of the coefficient of variation). These tests do not apply in our setting because they make two assumptions that would not be valid: (1) observations are independent across samples; and (2) observations are normally distributed.

The class of branching processes under consideration is defined in Section 2. Although this work was motivated by a problem that arises from cell biology, we consider a process that is more broadly applicable because our procedure works identically under a more general set of assumptions about the offspring and lifespan distributions, and generalization comes at no cost. In Section 3, we study the asymptotic behavior of the coefficient of variation of the population size when the immigration rate is constant and when it is time-dependent. The Markov case is treated analytically and the non-Markov case is investigated numerically. We develop the test in Section 4. In Section 5, we present results from simulation studies in which we investigate the performance of the test. These studies show that the test possesses adequate power under a variety of alternative hypotheses. An application to a real data set on the dynamics of leukemia is presented in Section 6. The analysis reveals that the immigration rate of normal (non-leukemic) cells into the blood changed over time. Concluding remarks are offered in Section 7. Technical details are provided in the Appendix.

2. Age-dependent branching processes with time-inhomogeneous immigration

2.1. The general process

Without loss of generality, the process begins at $t = 0$ with zero cells. Let $\{T_j\}_{j=1}^{\infty}$ be a sequence of time points at which I_j new cells (thereafter referred to as immigrants) arrive in the population, where $\{I_j\}_{j=1}^{\infty}$ is a collection of independent and identically distributed, integer-valued random variables (r.v.). Write $\gamma = E(I_j)$, $\gamma_2 = E\{I_j(I_j - 1)\}$, and $g(s) = E(s^{I_j})$. The immigration process $P(t) = \sum_{j=1}^{\infty} \mathbf{1}_{\{T_j \le t\}}$ is assumed to be a non-homogeneous Poisson process with instantaneous and cumulative rates $r(t)$ and $R(t) = \int_0^t r(u)du$. When $r(t) = r$ for every $t \ge 0$, $P(t)$ reduces to a standard time-homogeneous Poisson process.

Upon completion of its lifespan, every cell of the population, including immigrants, produces a random number ξ of offspring of age zero. Write $p_k = \mathrm{pr}(\xi = k)$, $k = 0, 1, \ldots$, for the distribution of ξ. Let $q(s) = E(s^{\xi})$, $|s| \le 1$, denote its probability generating function, and put $m = E(\xi)$ and $m_2 = E\{\xi(\xi - 1)\}$. Applications to cell biology are primarily concerned with the special case $q(s) = p_0 + p_2 s^2$, in which cells may either die with probability p_0 or divide into two cells with probability $p_2 = 1 - p_0$. The duration of the lifespan is described by a non-negative r.v. τ with cumulative distribution function $G(t) = \mathrm{pr}(\tau \le t)$, assumed non-lattice and satisfying $G(0+) = 0$. Write $\mu = E(\tau)$, assumed finite. Every cell behaves independently of every other cell. Finally, assume that $\gamma_2 < \infty$, $m_2 < \infty$, and $\mu_2 < \infty$. The distributions $G(\cdot)$ and $q(\cdot)$ define a Bellman-Harris process embedded in the branching process with immigration.

2.2. A special case applied to cancer stem cell biology

Recent studies have supported the hypothesis that stem cells play a central role not only in the generation and maintenance of multicellular systems, but also in the development of several cancers. For example, they have been identified in several types of leukemia [31]. As it appeared clear that stem cells should be targeted by cancer therapy, understanding their properties, including their dynamics, has become of considerable interest to cancer scientists.

Stem cells are characterized by the unique combination of three features: (1) they can self-renew by producing daughter cells that retain their properties; (2) they can generate multicellular lineages; and (3) they are able to maintain survival of these lineages. They also tend to be rare and are not always experimentally detectable, making the study of their behavior challenging.

The lack of direct observation on stem cells can be attenuated by modeling their contribution to disease progression via an immigration process that describes their influx into the pool of observable cells as they differentiate. The intensity of this influx of newly differentiated cells may vary over time,

a feature that can be captured by allowing the immigration process to be time-inhomogeneous. Upon completing their lifespan, differentiated cells divide into two differentiated cells with probability p_2 or die with probability $p_0 = 1 - p_2$. The duration of the lifespan is often assumed to have a gamma distribution, but other choices are possible (e.g., a log-normal distribution).

3. The coefficient of variation and its asymptotic behavior

3.1. The general case

Let $Z(t)$ denote the population size at time t. For every $t \geq 0$ and $|s| \leq 1$, put

$$\Psi(t;s) = E\{s^{Z(t)} \mid Z(0) = 0\}$$

for the probability generating function of $Z(t)$. It has the following expression:

$$\Psi(t;s) = \exp\left\{-\int_0^t r(t-u)[1 - g(F(u;s))]du\right\}, \tag{3.1}$$

where $\Psi(0,s) = 1$, and where $F(t;s)$, $t \geq 0$, $|s| \leq 1$, satisfies

$$F(t;s) = \int_0^t q(F(t-u;s))dG(u) + s\{1 - G(t)\}, \tag{3.2}$$

with the initial condition $F(0;s) = s$ [35]. Define $A(t) = \partial F(t;s)/\partial s|_{s=1}$ and $B(t) = \partial^2 F(t;s)/\partial s^2|_{s=1}$. These functions are the first and second order factorial moments of the embedded Bellman-Harris process started from a single cell at time $t = 0$. It follows from eqn. (3.2) that $A(t)$ and $B(t)$ are solutions to the renewal-type equations

$$A(t) = m\int_0^t A(t-u)dG(u) + 1 - G(t), \tag{3.3}$$

and

$$B(t) = m\int_0^t B(t-u)dG(u) + m_2\int_0^t A(t-u)^2 dG(u), \tag{3.4}$$

with the initial conditions $A(0) = 1$ and $B(0) = 0$ [1].

Define the first and second order moments of $Z(t)$: $M(t) = E\{Z(t) \mid Z(0) = 0\}$, $M_2(t) = E\{Z(t)\{Z(t)-1\} \mid Z(0) = 0\}$, $V(t) = \mathrm{Var}\{Z(t) \mid Z(0) = 0\} = M_2(t) + M(t)\{1 - M(t)\}$, and let $C_v(t) = V(t)^{1/2}/M(t)$ denote the coefficient of variation of $Z(t)$. We deduce from eqn. (3.1) that $M(t)$ and $M_2(t)$ take the expressions

$$M(t) = \gamma\int_0^t r(t-u)A(u)du, \tag{3.5}$$

and

$$M_2(t) = \gamma \int_0^t r(t-u)B(u)du + \left\{\gamma \int_0^t r(t-u)A(u)du\right\}^2 + \gamma_2 \int_0^t r(t-u)A(u)^2 du, \tag{3.6}$$

with initial conditions $M(0) = 0$ and $M_2(0) = 0$. Eqns. (3.5) and (3.6) imply directly that:

$$V(t) = \gamma \int_0^t r(t-u)B(u)du + \gamma_2 \int_0^t r(t-u)A(u)^2 du + M(t). \tag{3.7}$$

Let α denote the Malthusian parameter of the embedded Bellman-Harris process. Assuming it exists, α is the solution to the equation

$$m \int_0^\infty e^{-\alpha u} dG(u) = 1.$$

The process is said to be sub-critical if $\alpha < 0$, critical if $\alpha = 0$, and super-critical if $\alpha > 0$.

Define $\tilde{G}(t) = m \int_0^t e^{-\alpha u} dG(u)$ and $\tilde{\mu} = \int_0^\infty u d\tilde{G}(u)$. Application of renewal theory to eqns. (3.3) and (3.4) gives $A(t) = 1$ if $\alpha = 0$ and $A(t) \sim K_A e^{\alpha t}$ if $\alpha \neq 0$, $t \geq 0$, where $K_A = (m-1)/\alpha m \tilde{\mu}$, and

$$B(t) \sim \begin{cases} K_{B1} e^{\alpha t} & \text{if } \alpha < 0 \\ K_{B2} t & \text{if } \alpha = 0 \\ K_{B3} e^{2\alpha t} & \text{if } \alpha > 0, \end{cases}$$

where $K_{B1} = -m_2 K_A^2 / m\alpha\tilde{\mu}$, $K_{B2} = m_2/\mu$, and $K_{B3} = m_2 K_A^2 \tilde{G}(2\alpha)/\{1 - m\tilde{G}(2\alpha)\}$ [1].

Additional constants that will appear in the limit of the coefficient of variation include $\overline{A} = \int_0^\infty A(x)dx$ and $\overline{B} = \int_0^\infty B(x)dx$, which are both finite when $\alpha < 0$. We also define $\overline{E}_1(\alpha) = \int_0^\infty e^{-\alpha x}(1+x)^{-1}dx$, and $\widehat{A}_\rho = \int_0^\infty e^{-\rho u} A(x)dx$. Notice that $\overline{E}_1(\alpha) < \infty$ if $\alpha > 0$, and $\widehat{A}_\rho < \infty$ if $\rho > \alpha$.

To simplify the presentation, we discuss the asymptotic behavior of the expectation and variance of the process in Theorems A.1, A.2 and A.3 which have been placed in the Appendix. The asymptotic behavior of the coefficient of variation is easily deduced from these theorems. Beginning with the time-homogeneous case, we have:

Theorem 3.1 (Time-homogeneous Poisson process). *Assume that* $r(\cdot) \equiv r$. *Then* $\lim_{t\to\infty} C_v(t) = c_0$, *where*

$$c_0 = \begin{cases} \sqrt{\frac{\gamma(\overline{A}+\overline{B})+\gamma_2\overline{A^2}}{r\gamma^2\overline{A}^2}} & \text{if } \alpha < 0 \\ \sqrt{\frac{m_2}{2r\gamma\mu}} & \text{if } \alpha = 0 \\ \sqrt{\frac{\alpha(\gamma K_{B3}+\gamma_2 K_A^2)}{2r\gamma^2 K_A^2}} & \text{if } \alpha > 0. \end{cases}$$

When the immigration process is time-inhomogeneous, the behavior of $C_v(t)$ differs from that exhibited in the time-homogeneous case. Using c as a generic notation to denote a positive constant that appears in the limit of $C_v(t)$, and which differs in all cases, we have:

Theorem 3.2 (Time-inhomogeneous Poisson process).

Case 1. If $r(t) = r/(1+t)$, *then*

$$C_v(t) \sim \begin{cases} c\sqrt{t} & \text{if } \alpha < 0 \\ c\sqrt{\frac{t}{\log t}} & \text{if } \alpha = 0 \\ \sqrt{\frac{\gamma K_{B3} + \gamma_2 K_A^2}{r\gamma^2 K_A^2 \overline{E}_1(\alpha)}} & \text{if } \alpha > 0. \end{cases}$$

Case 2. If $r(t) = rt^\theta$, $\theta > -1$, *then*

$$C_v(t) \sim \begin{cases} ct^{-\theta/2} & \text{if } \alpha \leq 0 \\ \sqrt{\frac{\alpha^{\theta+1}(\gamma K_{B3} + \gamma_2 K_A^2)}{2r\gamma^2 K_A^2 \Gamma(\theta+1)}} & \text{if } \alpha > 0. \end{cases}$$

Case 3. If $r(t) = re^{\rho t}$, $\rho > 0$, *then*

$$C_v(t) \sim \begin{cases} \sqrt{\frac{(\alpha-\rho)^2(\gamma K_{B3} + \gamma_2 K_A^2)}{r\gamma^2 K_A^2 (2\alpha - \rho)}} & \text{if } \rho < \alpha \\ ct^{-1} & \text{if } \rho = \alpha \\ ce^{-(\rho-\alpha)t} & \text{if } \alpha < \rho < 2\alpha \\ c\sqrt{t}e^{-\alpha t} & \text{if } \rho = 2\alpha \\ ce^{-\rho t/2} & \text{if } \rho > 2\alpha. \end{cases}$$

Taken together, these results suggest the following conclusions. If the immigration process is time-homogeneous, we see from Theorem 3.1 that $\log C_v(t)$ converges to a constant $\log c_0$ as $t \to \infty$. If it is time-inhomogeneous, Theorem 3.2 indicates that $\log C_v(t) \to \pm\infty$ as $t \to \infty$ in most of the considered cases, except (e.g.) for the exponential rate when $0 < \rho < \alpha$, where it converges to a constant that differs from $\log c_0$. Moreover, when it diverges, $\log C_v(t)$ is, in most cases, asymptotically equivalent to an affine function of $h(t)$, $\log C_v(t) \sim a_0 + a_1 h(t)$, where $h(t)$ is a function that depends solely on time (e.g., $h(t) = t$ or $h(t) = \log t$ or $h(t) = \log\log t$) and no other parameters. We will use this property when constructing our test.

3.2. The Markov case

The moments of $Z(t)$ are available in closed-form when the process is Markov and the immigration process is time-homogeneous; that is, when $G(t) = 1 - e^{-t/\mu}$ and $r(t) = r$, for every $t \geq 0$. Specifically, the expectation and variance of the population size are

$$M(t) = \begin{cases} \frac{\gamma r}{\alpha}(e^{\alpha t} - 1) & \text{if } \alpha \neq 0 \\ \gamma r t & \text{if } \alpha = 0, \end{cases}$$

and

$$V(t) = \begin{cases} \frac{\gamma r m_2 + \gamma_2 r \alpha \mu}{2\alpha^2 \mu}(e^{2\alpha t} - 1) - \frac{\gamma r}{\alpha}(\frac{m_2}{\alpha\mu} - 1)(e^{\alpha t} - 1) & \text{if } \alpha \neq 0 \\ \frac{\gamma r m_2}{2\mu} t^2 + r(\gamma_2 + \gamma)t & \text{if } \alpha = 0. \end{cases}$$

We deduce immediately that

$$C_v(t) \quad = \quad \begin{cases} \sqrt{\frac{\gamma m_2 + \gamma_2 \alpha \mu}{2\mu\gamma^2 r} \frac{e^{\alpha t}+1}{e^{\alpha t}-1} + \frac{\alpha}{\gamma r} \frac{1 - m_2/\alpha\mu}{e^{\alpha t}-1}} & \text{if } \alpha \neq 0 \\ \sqrt{\frac{m_2}{2\mu\gamma r} + \frac{\gamma_2 + \gamma}{r\gamma^2 t}} & \text{if } \alpha = 0. \end{cases}$$

The expectation and variance are therefore asymptotically equivalent to

$$M(t) \quad \sim \quad \begin{cases} -\frac{r\gamma}{\alpha} & \text{if } \alpha < 0 \\ r\gamma t & \text{if } \alpha = 0 \\ \frac{r\gamma}{\alpha} e^{\alpha t} & \text{if } \alpha > 0, \end{cases}$$

and

$$V(t) \quad \sim \quad \begin{cases} -\frac{r\gamma}{\alpha} + \frac{r(\gamma m_2 - \gamma_2 \alpha \mu)}{2\mu\alpha^2} & \text{if } \alpha < 0 \\ \frac{r\gamma m_2}{2\mu} t^2 & \text{if } \alpha = 0 \\ \frac{r}{2\mu\alpha^2}(\gamma m_2 + \gamma_2 \alpha \mu) e^{2\alpha t} & \text{if } \alpha > 0. \end{cases}$$

Finally, we deduce that $\lim_{t\to\infty} C_v(t) = c_0$, where

$$c_0 = \begin{cases} \sqrt{-\frac{\gamma_2\alpha\mu + \gamma m_2}{2r\mu\gamma^2} + \frac{\alpha}{\gamma r}(1 - \frac{m_2}{\alpha\mu})} & \text{if } \alpha < 0 \\ \sqrt{\frac{m_2}{2r\mu\gamma}} & \text{if } \alpha = 0 \\ \sqrt{\frac{\gamma m_2 + \gamma_2 \alpha \mu}{2r\mu\gamma^2}} & \text{if } \alpha > 0, \end{cases}$$

which is consistent with Theorem 3.1. Moreover,

$$C_v(t) - c_0 = \begin{cases} O(e^{-|\alpha| t}) & \text{if } \alpha \neq 0 \\ O(t^{-1}) & \text{if } \alpha = 0, \end{cases} \tag{3.8}$$

and convergence to c_0 occurs quickly over time in all cases.

3.3. Numerical investigations in the non-Markov case

We present results from numerical simulations that further illustrate the behavior of the coefficient of variation of the population size with various immigration rates, including some that were considered in Theorem 3.2.

The population size process $Z(t)$ $(t \geq 0)$ was simulated by assuming that: (i) the lifespan of every cell has a gamma (non-exponential) distribution with mean 24 and variance 48; and (ii) upon completion of its lifespan, every cell either divides with probability p_2 or dies with probability $p_0 = 1 - p_2$. We considered different values of p_0 to run simulations with sub- $(p_0 > 0.5)$, super- $(p_0 < 0.5)$, and critical $(p_0 = 0.5)$ processes.

We also considered various rates for the immigration process, including the time-homogeneous rate $(r(\cdot) \equiv r)$ and the following time-inhomogeneous rates:

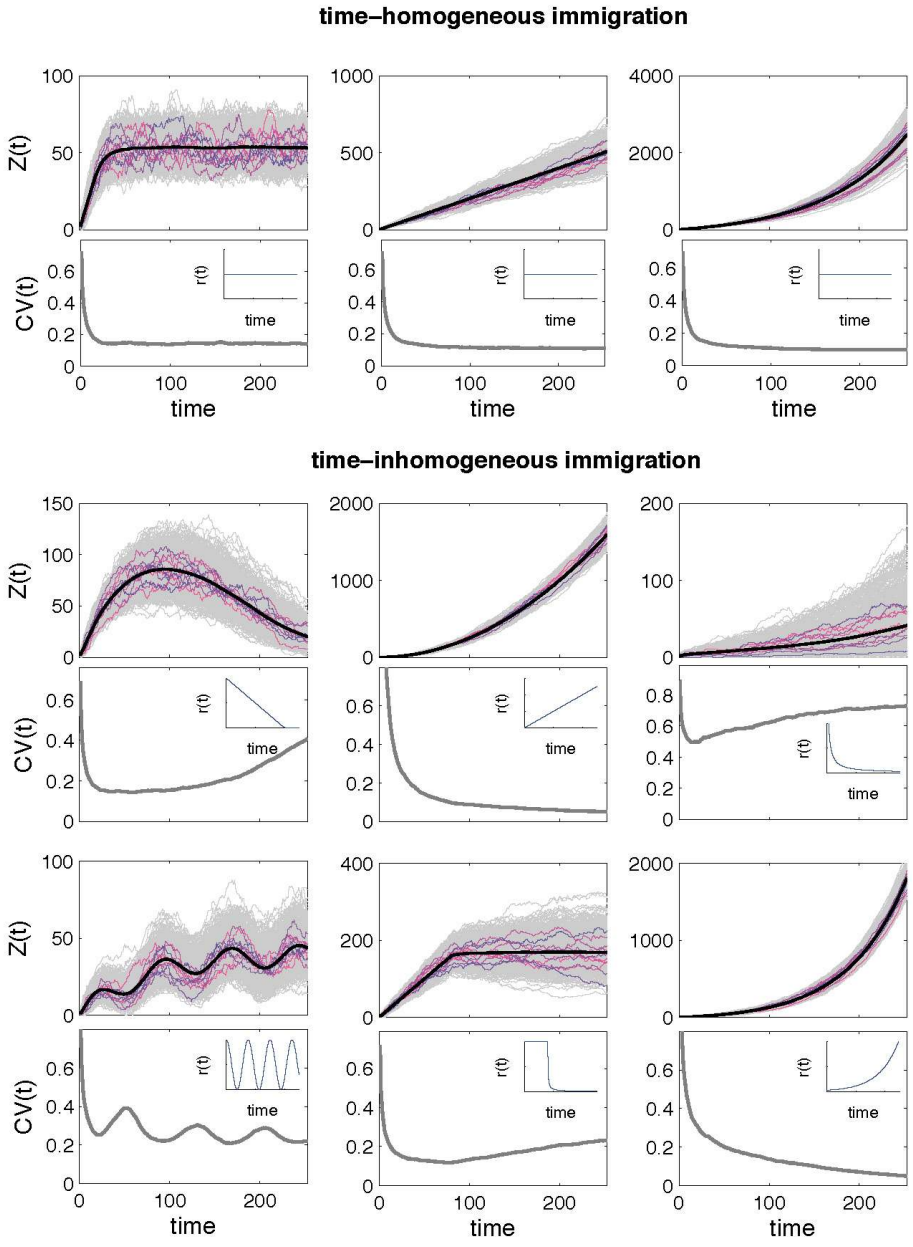

FIG 1. *Results from simulations. For each scenario we report: 1,000 simulated sample paths (gray and violet lines), their empirical mean (black line) and coefficient of variation (CV(t)), as well as the immigration rate (r(t)). The panels on the top were obtained with time-homogeneous immigration processes and those below were obtained with various time-dependent immigration processes. The immigration rate is displayed in the subplot embedded in the plot of the coefficient of variation.*

- $r(t) = (r + \theta t) \vee 0$: the immigration rate increases or decreases affinely over time and is constrained to remain positive when $\theta < 0$.
- $r(t) = r/(1 + t)$: the immigration rate decreases gradually to 0 over time.
- $r(t) = r\{1 + \cos(\theta t)\}$: the immigration rate oscillates over time between 0 and $2r$.
- $r(t) = \begin{cases} r & \text{if } t \in [0, t_0] \\ \{r + \theta(t - t_0)\} \vee 0 & \text{if } t > t_0. \end{cases}$
  Here, the immigration rate remains constant between times 0 and t_0, and increases or decreases affinely thereafter.
- $r(t) = re^{\theta t}$: the immigration rate increases exponentially fast over time; this scenario could describe immigration of malignant cells from tissues (e.g., bone marrow) in which they multiply exponentially fast.

We simulated the time-inhomogeneous Poisson process using Lewis and Shedler (1979)'s acceptance-rejection method [36]. A single immigrant entered the population at every time T_j (that is, $\text{pr}(I_j = 1) = 1$, $j = 1, 2, \ldots$).

We simulated 1,000 runs of the branching process with immigration over the time interval $[0, 250]$, and computed the empirical mean, variance and coefficient of variation of these simulations at multiple time points. The results are shown in Figure 1. The plots in the left column were obtained with sub-critical processes ($p_0 > 0.5$); those in the center were obtained with critical processes ($p_0 = 0.5$); and those on the right were obtained with super-critical processes ($p_0 < 0.5$). In the first two rows, we used a time-homogeneous immigration process. The results displayed in the next four rows were obtained with time-inhomogeneous immigration processes. The immigration rate was different in all cases, as indicated in the figure.

When the immigration process was time-homogeneous, the coefficient of variation of $Z(t)$ decreased over time to c_0. Convergence was quick and virtually occurred within the time interval $[0, 40]$. When the immigration process is time-inhomogeneous, the simulations indicate that the coefficient of variation was time-dependent. Thus, these simulations corroborate results stated in Theorem 3.2. The magnitude of the change in the coefficient of variation differed across scenarios.

4. An asymptotic test of homogeneity of the immigration process

We now develop a test of the null hypothesis:

$$H_0 : \text{“}r(\cdot) \equiv r\text{”}$$

that the rate of the immigration process is a constant function of time, and we will test H_0 against the general alternative hypothesis $H_1 = \overline{H}_0$. The proposed procedure applies when several, independent realizations of the process are observed at discrete points in time. This type of experimental design is commonly used in biology.

Thus, let $\{Z_i(t)\}_{t \geq 0}$, $i = 1, \ldots, n$, denote n independent and identically distributed (i.i.d.) copies of the process $\{Z(t)\}_{t \geq 0}$. Assume that each of them is

observed at m ($m < \infty$) discrete time points $t_1, \ldots, t_m$ with $0 < t_1 < \cdots < t_m < \infty$. Write $\mathbf{t} = (t_1, \ldots, t_m)$. We note that $t_1 \to \infty$ implies that $t_j \to \infty$ ($j = 1, \ldots, m$).

We could construct a test of H_0 against the alternative hypothesis H_1 by fitting a branching process with time-inhomogeneous immigration to the data and assessing the significance of the association of the immigration rate with time. This approach would first require specifying a family of distributions for the lifespan and a functional form for the rate of the immigration process. We would next fit the postulated model and construct a p-value to decide whether H_0 should be rejected. Estimation methods are not yet available for age-dependent branching processes with immigration, but could arguably be developed (e.g., by adapting an existing pseudo-likelihood approach proposed for age-dependent branching processes [6, 37]). Here, we consider a different approach that requires none of these steps and assumptions to be made. The developed test only requires existence of a model in the class of branching processes defined in Section 2.1 that can describe the data. It also requires fitting a linear regression model to the empirical coefficients of variation.

The construction of our test rests on Theorem 3.1, which ensures, if H_0 is true, that the coefficient of variation of $Z(t)$ will remain virtually constant between all times of observation, provided that t_1 is sufficiently large. Thus, we propose to test H_0 by checking whether the empirical coefficients of variation computed using the samples $\{Z_i(t_j)\}_{i=1}^n, j = 1, \ldots, m$, differ significantly between time points. The comparison is accomplished via linear regression.

For every $t \geq 0$, define the kth central moment $M_{(k)}(t) = E[\{Z(t) - M(t)\}^k]$, assumed finite for $k = 1, \ldots, 4$. Let

$$\hat{m}(t) = n^{-1} \sum_{i=1}^{n} Z_i(t),$$

$$\hat{v}(t) = (n-1)^{-1} \sum_{i=1}^{n} \{Z_i(t) - \hat{m}(t)\}^2$$

and

$$\hat{c}_v(t) = \sqrt{\hat{v}(t)}/\hat{m}(t) \tag{4.1}$$

denote the sample mean, variance, and coefficient of variation. Using a result from [39], we have that

$$n^{1/2}\{\hat{c}_v(t) - C_v(t)\} \xrightarrow{\mathcal{D}} \mathcal{N}\{0, \sigma^2(t)\},$$

where

$$\sigma^2(t) = C_v(t)^4 - M_{(3)}(t)M(t)^{-3} + M_{(4)}(t)\{4M(t)^2V(t)\}^{-1} - C_v(t)^2 4^{-1}.$$

This convergence uses a Central Limit Theorem for i.i.d. random variables [38], which applies here because $\{Z_i(t)\}_{i=1}^n$ are i.i.d. by assumption. It also requires

that $M_{(4)}(t) < \infty$. Hence, for any real-valued function $L(\cdot)$ with derivative $L'(x) \neq 0, x > 0$, we have that

$$L(\hat{c}_v(t)) = L(C_v(t)) + n^{-1/2}\varepsilon(t) + o_p(n^{-1/2}), \tag{4.2}$$

where

$$\varepsilon(t) \sim \mathcal{N}\left\{0, L'(C_v(t))^2\sigma^2(t)\right\}.$$

Here, the function $L(\cdot)$ refers to any transformation of $C_v(t)$ that may make it a diverging function of time, should the null hypothesis be violated. Relevant examples include $L(u) = \log u$ or $L(u) = u$, as discussed at the end of Section 3.1. Write $\Sigma_{\mathbf{t}} = \mathrm{Var}[(\varepsilon(t_1), \ldots, \varepsilon(t_m))]$ and let $\Sigma_\infty = \lim_{t_1 \to \infty} \Sigma_{\mathbf{t}}$, assuming the limit exists under H_0.

When $r(\cdot) \equiv r$, Theorem 3.1 and eqn. (4.2) entail that

$$L(\hat{c}_v(t)) = L(c_0) + n^{-1/2}\varepsilon(t) + o(1) + o_p(n^{-1/2}), \tag{4.3}$$

where $o(1) \to 0$ as $t \to \infty$. Thus, $L(\hat{c}_v(t))$ is virtually centered about $L(c_0)$ under H_0 if t is sufficiently large, and we saw in Sections 3.2 and 3.3 that this occurs quickly. From Theorem 3.2, we expect that this is not the case when $r(\cdot)$ is time-dependent.

In order to construct our test statistic, we now formulate the linear model

$$L(\hat{c}_v(t_j)) = x_j^{\mathrm{T}}\theta + \eta(t_j) \qquad (j = 1, \ldots, m) \tag{4.4}$$

where

$$x_j = x_j(t_j) := \{1 \; h_1(t_j) \; \cdots \; h_{p-1}(t_j)\}^{\mathrm{T}}$$

is a vector of covariates defined using a given set of functions $h_1(\cdot), \ldots, h_{p-1}(\cdot)$, for some $p \geq 2$, where $\theta = (\theta_1, \ldots, \theta_p)^{\mathrm{T}}$ is a p-dimensional parameter vector, and where the r.v. $\eta(t_j)$ describes the error term of the model. The error terms $\eta(t_j)$, $j = 1, \ldots, m$, are not formally centered about zero; however, when H_0 is true, it follows from Theorem 3.1 that their expectation converges to zero as $t_j \to \infty$.

When H_0 is true and when t_1 is sufficiently large, we expect from eqn. (4.3) that Model (4.4) captures the behavior of $L(\hat{c}_v(t))$ with $\theta \simeq \theta^*$, where

$$\theta^* = \{L(c_0), 0, \ldots, 0\}^{\mathrm{T}},$$

because all coefficients associated with time should be zero. The functions $h_1(\cdot)$, $\ldots, h_{p-1}(\cdot)$ are chosen so Model (4.4) can detect a change in $L(\hat{c}_v(t))$ over time when H_1 is true. With reasonably chosen $h_k(\cdot)$, we expect the relationship (4.4) to hold with $\theta \neq \theta^*$ under H_1 because $L(\hat{c}_v(t_i))$ should now be time-dependent. Thus, the gist of the proposed test is to assess H_0 by verifying whether $\theta = \theta^*$.

Define

$$\hat{L}_{cv}(\mathbf{t}) = \{L(\hat{c}_v(t_1)), \ldots, L(\hat{c}_v(t_n))\}^{\mathrm{T}},$$
$$L_{cv}(\mathbf{t}) = \{L(C_v(t_1)), \ldots, L(C_v(t_n))\}^{\mathrm{T}},$$

and $X = (x_1, \ldots, x_m)^{\mathrm{T}}$. Assume that

Assumption 1. rank $X = p$.

Assumption 1 implies that the matrix $X^{\mathrm{T}}X$ is invertible. Let

$$\hat{\theta} = (X^{\mathrm{T}}X)^{-1}X^{\mathrm{T}}\hat{L}_{cv}(\mathbf{t})$$

denote the least squares estimator of θ for Model (4.4), and define

$$\theta^*_{\mathbf{t}} = (X^{\mathrm{T}}X)^{-1}X^{\mathrm{T}}L_{cv}(\mathbf{t}). \tag{4.5}$$

As $n \to \infty$, we have that $\hat{L}_{cv}(\mathbf{t}) \xrightarrow{P} L_{cv}(\mathbf{t})$, $\hat{\theta} \xrightarrow{P} \theta^*_{\mathbf{t}}$, and

$$\begin{aligned} \sqrt{n}(\hat{\theta} - \theta^*_{\mathbf{t}}) &= (X^{\mathrm{T}}X)^{-1}X^{\mathrm{T}}\{\hat{L}_{cv}(\mathbf{t}) - L_{cv}(\mathbf{t})\} \\ &\xrightarrow{\mathcal{D}} \mathcal{N}(\mathbf{0}, V_{\mathbf{t}}), \end{aligned} \tag{4.6}$$

where

$$V_{\mathbf{t}} = (X^{\mathrm{T}}X)^{-1}X^{\mathrm{T}}\Sigma_{\mathbf{t}}X(X^{\mathrm{T}}X)^{-1}.$$

Under H_0, when both n and t_1 increase at appropriate rates, we have that

$$\sqrt{n}(\hat{\theta} - \theta^*) \xrightarrow{\mathcal{D}} \mathcal{N}(\mathbf{0}, V_\infty),$$

where

$$V_\infty = \lim_{t_1 \to \infty} V_{\mathbf{t}} = (X^{\mathrm{T}}X)^{-1}X^{\mathrm{T}}\Sigma_\infty X(X^{\mathrm{T}}X)^{-1}.$$

Let $\rho(t)$ denote the rate at which $C_v(t)$ converges to c_0 as $t \to \infty$ when H_0 is true; that is, $\rho(t)$ is such that

$$C_v(t) - c_0 = O(\rho(t)).$$

For example, in the Markov case, we showed that

$$\rho(t) = \begin{cases} e^{-|\alpha|t} & \text{if } \alpha \neq 0 \\ t^{-1} & \text{if } \alpha = 0 \end{cases} \tag{4.7}$$

(see eqn. (3.8)). Since θ^* can be expressed as

$$\theta^* = (X^{\mathrm{T}}X)^{-1}X^{\mathrm{T}}L(c_0)\mathbf{1}_p,$$

where $\mathbf{1}_p$ is a $p \times 1$ vector with all entries equal to 1, it follows from eqn. (4.5) and a Taylor series expansion that

$$\begin{aligned} \theta^*_{\mathbf{t}} - \theta^* &= (X^{\mathrm{T}}X)^{-1}X^{\mathrm{T}}[L_{cv}(\mathbf{t}) - L(c_0)\mathbf{1}_p] \\ &= (X^{\mathrm{T}}X)^{-1}X^{\mathrm{T}}\left[L'(c_0)\{C_v(\mathbf{t}) - c_0\mathbf{1}_p\} + o(\|C_v(\mathbf{t}) - c_0\mathbf{1}_p\|)\right]. \end{aligned}$$

Thus, when H_0 holds, we deduce that

$$\|\theta^*_{\mathbf{t}} - \theta^*\| = O\left[\max_{j=1,\ldots,m} \rho(t_j)\right]. \tag{4.8}$$

Although converging to 0 under H_0 as $t_1 \to 0$, the residual difference between $\theta^*_{\mathbf{t}}$ and θ^* could be detected if n is large enough, even if H_0 is true. Thus, to impose conditions controlling inflation of the type-1 error rate of our test, we further assume that the study is designed such that:

Assumption 2. If H_0 holds true, then $n = o[\{\max_{j=1,\ldots,m} \rho(t_j)\}^{-2}]$.

This second assumption prevents the sample size n from being too large relative to the time points t_j, $j = 1, \ldots, m$. It is needed to eliminate the asymptotic bias that may persist in $\hat{\theta}$ under H_0 as $n \to \infty$ as a result of the fact that $C_v(t)$ is not equal to c_0 when t is finite. It is verified if the process has been running for a sufficiently long time before the first observations are made. For example, in the application presented in Section 6, Assumption 2 is expected to be satisfied by the population of normal cells since hemopoiesis, the process of blood cell production, had reached steady state before leukemia cells were inoculated. We note that the assumption pre-assumes that the unit of time remains fixed as n increases.

To further understand the implications of Assumption 2, we can consider the Markov case for which $\rho(t)$ is given in eqn. (4.7). Hence, we have that $\max_{j=1,\ldots,m} \rho(t_j) = \rho(t_1)$, and Assumption 2 is satisfied in this case if n increases with t_1 at the following rate:

$$n = \begin{cases} o\left(e^{2|\alpha|t_1}\right) & \text{if } \alpha \neq 0 \\ o\left(t_1^2\right) & \text{if } \alpha = 0. \end{cases}$$

Therefore, in the Markov case, the impact of increasing the sample size on type-1 errors may be overcome by a moderate increase in t_1. We note that sample sizes are rarely large in biological experiments, such that the validity of Assumption 2 may not be of concern as long as the process is not observed too shortly after the population started to grow.

When H_0 is true, Assumption 2 together with eqns. (4.3), (4.6) and (4.8) imply that

$$\begin{aligned} \sqrt{n}(\hat{\theta} - \theta^*) &= \sqrt{n}(\hat{\theta} - \theta_{\mathbf{t}}^*) + \sqrt{n}(\theta_{\mathbf{t}}^* - \theta^*) \\ &\xrightarrow{\mathcal{D}} \mathcal{N}(\mathbf{0}, V_\infty), \end{aligned}$$

as $n \to \infty$. For every $k = 1, \ldots, q$, let $\hat{\theta}_k$ denote the kth entry of $\hat{\theta}$. Write $\hat{\theta}_{2:p} = (\hat{\theta}_2, \ldots, \hat{\theta}_p)^{\mathrm{T}}$, and let $V_{\mathbf{t}}^{(2:p)}$ denote the sub-matrix of $V_{\mathbf{t}}$ that corresponds to the asymptotic variance-covariance matrix of $\hat{\theta}_{2:p}$. Define the Wald statistic

$$W = n\hat{\theta}_{2:p}^{\mathrm{T}}\{V_{\mathbf{t}}^{(2:p)}\}^{-1}\hat{\theta}_{2:p}.$$

The above derivations yield immediately the following result:

Theorem 4.1. *Assume that Assumptions 1–2 hold. Then* $W \xrightarrow{\mathcal{D}_{H_0}} \chi^2_{p-1}$ *as* $n \to \infty$ *where* χ^2_{p-1} *is a chi-squared distributed random variable with* $p-1$ *degrees of freedom.*

To implement the test, we must estimate the variance-covariance matrix $\Sigma_{\mathbf{t}}$ in order to compute the matrix $V_{\mathbf{t}}^{(2:p)}$ used in the expression for W. In simulation studies and in analyses of our experimental data on the progression of leukemia, we have used a bootstrap estimator [32, 33]. We generate B independent samples

$\{Z_i^{*,b}(t), t \geq 0\}_{i=1}^n$ by sampling with replacement from $\{Z_i(t), t \geq 0\}_{i=1}^n$, and calculate $\hat{c}_v^{*,b}(t_j)$, $j = 1, \ldots, m$, according to eqn. (4.1) based on the bootstrap sample $\{Z_i^{*,b}(t_j)\}_{i=1}^n$, $b = 1, \ldots, B$. We set $B = 5{,}000$ in our simulations and application. Writing

$$L^{*,b}(\mathbf{t}) = \left(L^{*,b}(\hat{c}_v^{*,b}(t_1)), \ldots, L^{*,b}(\hat{c}_v^{*,b}(t_m))\right)^{\mathrm{T}},$$

we finally approximate $\Sigma_{\mathbf{t}}$ by

$$\hat{\Sigma}_{\mathbf{t}} = \frac{n}{B-1} \sum_{b=1}^{B} \left\{ L^{*,b}(\mathbf{t}) - \frac{1}{B} \sum_{b=1}^{B} L^{*,b}(\mathbf{t}) \right\} \left\{ L^{*,b}(\mathbf{t}) - \frac{1}{B} \sum_{b=1}^{B} L^{*,b}(\mathbf{t}) \right\}^{\mathrm{T}}.$$

Shao [34] proposed a simple modification of this bootstrap variance estimator based on the idea of truncation. This estimator achieves consistency under broader assumptions that hold in the present setting if $M_{(4)}(t) < \infty$.

Once an estimator for $\Sigma_{\mathbf{t}}$ has been chosen, the test is implemented by rejecting H_0 at the significance level δ if $W \geq \chi^2_{p-1}(1-\delta)$, where $\chi^2_{p-1}(1-\delta)$ is the $100(1-\delta)$th percentile of a chi-squared distribution with $p-1$ degrees of freedom.

In practice, the processes $\{Z_i(t)\}_{t \geq 0}$, $i = 1, \ldots, n$, need not all be observed under identical experimental conditions. For example, in our leukemia experiment, we have two groups of observations, each corresponding to a particular sampling scheme. Specificities of the experimental design may be accommodated in the test by including appropriate covariates in the vector of predictors x_j. The test should then reject H_0 if the resulting test statistic W is greater than or equal to the percentile of a chi-squared distribution with p_0 degrees of freedom where p_0 denotes the number of predictors included in x_j that are associated with time, t_j. See Section 6 for specific examples.

To obtain the power function of the test, we expand W as

$$\begin{aligned} W &= n(\hat{\theta}_{2:p} - \theta^*_{\mathbf{t},2:p})^{\mathrm{T}} \{V_{\mathbf{t}}^{(2:p)}\}^{-1} (\hat{\theta}_{2:p} - \theta^*_{\mathbf{t},2:p}) \\ &\quad + n(\hat{\theta}_{2:p} - \theta^*_{\mathbf{t},2:p})^{\mathrm{T}} \{V_{\mathbf{t}}^{(2:p)}\}^{-1} \theta^*_{\mathbf{t},2:p} \\ &\quad + n\theta^{*\mathrm{T}}_{\mathbf{t},2:p} \{V_{\mathbf{t}}^{(2:p)}\}^{-1} \theta^*_{\mathbf{t},2:p}. \end{aligned} \tag{4.9}$$

The first term in the right-hand side of eq. (4.9) converges in distribution under Assumption 1 to a chi-squared distributed random variable with $p-1$ degrees of freedom; the second term is asymptotically equivalent to $\sqrt{n}\, U^{\mathrm{T}} \{V_{\mathbf{t}}^{(2:p)}\}^{-T/2} \theta^*_{\mathbf{t},2:p}$, where U is a standard normal $(p-1) \times 1$ random vector. An asymptotic approximation to the power function of the test in large samples is

$$Q(\delta) \simeq \mathrm{pr}\left(U^{\mathrm{T}}U - 2\sqrt{n}\, U^{\mathrm{T}}\Delta > \chi^2_{p-1}(1-\delta) - n\Delta^{\mathrm{T}}\Delta\right),$$

where $\Delta = \{V_{\mathbf{t}}^{(2:p)}\}^{-T/2} \theta^*_{\mathbf{t},2:p}$ represents a standardized effect size defined as the departure from the null hypothesis expressed through the coefficients of the regression model (4.4) that are associated with time, which we have normalized

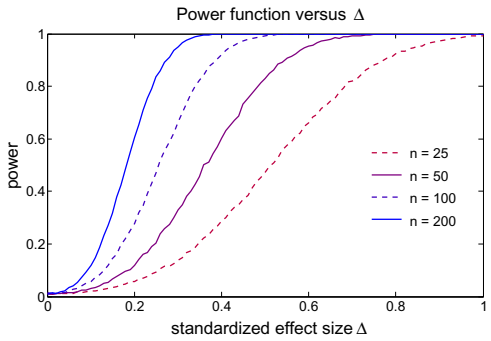

FIG 2. *Asymptotic power function* (Q) *plotted against* Δ *for various sample sizes* (n).

by the variability matrix $V_{\mathbf{t}}^{(2:p)}$. The approximation of the power by $Q(\delta)$ as a function of the standardized effect size is illustrated in Figure 2.

The power of the test may be affected by the choice of the regression function of Model (4.4), and one must carefully evaluate the benefits of increasing the number of functions $h_k(\cdot)$ included in the design matrix X in order to capture (potential) complex temporal patterns in the coefficient of variation. In our data analysis, the coefficient of variation appeared to increase linearly over time (for time points ≥ 6 days), such that building W with a regression Model (4.4) in which the regression function depends linearly on time should be an optimal choice. By increasing the flexibility of the regression function (e.g., allowing this function to depend quadratically on time), we increase the degrees of freedom of the asymptotic distribution of W, which could result in loss of power.

5. Simulation studies

We performed simulations to evaluate the level and power of the proposed test under a variety of immigration rates. The branching process used to generate the data assumed that $G(t)$ was a gamma distribution with mean 20 and variance 40. The offspring generating function was $q(s) = p_0 + (1 - p_0)s^2$, and we considered three values for p_0 to evaluate the test when the process is subcritical ($p_0 = 0.75$), critical ($p_0 = 0.5$) and supercritical ($p_0 = 0.35$ or 0.45). These parameter values are biologically plausible when time is measured in hours.

We also considered immigration rates with various shapes to study the level of the test when immigration is time-homogeneous and its power when it depends on time. The functions that define the immigration rates are included in Tables 1 and 2 which show the percentages of times the test rejected the null hypothesis in each scenario. We note that the layout of these tables mirrors that of Figure 1 (for example, in both the figure and tables, the top rows display results obtained with a time-homogeneous immigration process), and thus the reader can examine results from these simulation studies in light of the behavior of the process $Z(t)$ and its coefficient of variation.

TABLE 1

Percentage of simulated data sets for which the test rejected H_0 at the 5% nominal level as a function of the sample size and for different types of immigration rates. For each tested scenario, the table reports results based on test statistics constructed using $x_j = (1, t_j)'$ (linear) and $x_j = (1, t_j, t_j^2)'$ (quadratic), and with log-transformed coefficients of variation ($L(u) = \log u$)

time-homogeneous immigration process

	subcritical $p_0 = 0.75$ $r(t) = 2$		critical $p_0 = 0.5$ $r(t) = 2$		supercritical $p_0 = 0.35$ $r(t) = 2$	
sample size	linear	quadratic	linear	quadratic	linear	quadratic
25	6.9%	9.4%	6.7%	9.4%	7.6%	8.8%
50	7.1%	8.7%	6.6%	7.2%	7.9%	6.4%
100	5.6%	6.3%	5.8%	6.9%	9.4%	9.7%
200	5.4%	6.1%	6.8%	6.8%	9.8%	9.0%

time-inhomogeneous immigration process

	subcritical $p_0 = 0.75$ $r(t) = (2 - 0.01t) \vee 0$		critical $p_0 = 0.5$ $r(t) = 0.05t$		supercritical $p_0 = 0.35$ $r(t) = \frac{3}{2(1+t)}$	
sample size	linear	quadratic	linear	quadratic	linear	quadratic
25	100.0%	100.0%	24.8%	23.9%	11.9%	11.3%
50	100.0%	100.0%	40.1%	38.9%	17.4%	16.7%
100	100.0%	100.0%	67.6%	59.1%	25.5%	23.4%
200	100.0%	100.0%	92.9%	89.2%	43.7%	37.5%
	subcritical $p_0 = 0.75$ $r(t) = \frac{1}{2}\{1 + \cos(\frac{t}{12})\}$		critical $p_0 = 0.5$ $r(t) = \frac{2}{1+(t-80)\vee 0}$		supercritical $p_0 = 0.45$ $r(t) = \frac{1}{2}e^{0.015t}$	
sample size	linear	quadratic	linear	quadratic	linear	quadratic
25	7.2%	82.2%	40.0%	35.3%	80.0%	76.6%
50	8.9%	99.2%	63.6%	58.4%	99.1%	96.0%
100	9.5%	100.0%	91.7%	83.4%	100.0%	100.0%
200	13.2%	100.0%	100.0%	98.8%	100.0%	100.0%

For each scenario that was tested, we simulated 1,000 data sets of a given sample size (n). We considered different values of n : 25, 50, 100 and 200. The population size was observed every 12 hours from day 7 to day 10 (thus, $m = 7$), similar to the study design of our leukemia experiment. In Table 1, the test statistic was constructed with log-transformed coefficients of variation (that is, $L(u) = \log u$). In Table 2, we used untransformed coefficients of variation (that is, $L(u) = u$). In either case, we set $x_j = (1, t_j)^{\mathrm{T}}$ and $x_j = (1, t_j, t_j^2)^{\mathrm{T}}$, $j = 1, \ldots, 6$, to study the influence of the choice of x_j on performances.

TABLE 2

Percentage of simulated data sets for which the test rejected H_0 at the 5% nominal level as a function of the sample size and for different types of immigration rates. For each tested scenario, the table reports results based on test statistics constructed using $x_j = (1, t_j)'$ (linear) and $x_j = (1, t_j, t_j^2)'$ (quadratic). Here, we did not transformed the coefficients of variation ($L(u) = u$)

time-homogeneous immigration process

	subcritical $p_0 = 0.75$ $r(t) = 2$		critical $p_0 = 0.5$ $r(t) = 2$		supercritical $p_0 = 0.35$ $r(t) = 2$	
sample size	linear	quadratic	linear	quadratic	linear	quadratic
25	7.4%	9.9%	8.9%	12.3%	9.1%	11.6%
50	7.3%	6.8%	8.9%	8.8%	8.5%	9.1%
100	6.7%	6.6%	6.9%	7.0%	7.7%	6.9%
200	5.7%	6.4%	6.6%	7.6%	9.6%	10.2%

time-inhomogeneous immigration process

	subcritical $p_0 = 0.75$ $r(t) = (2 - 0.01t) \vee 0$		critical $p_0 = 0.5$ $r(t) = 0.05t$		supercritical $p_0 = 0.35$ $r(t) = \frac{3}{2(1+t)}$	
sample size	linear	quadratic	linear	quadratic	linear	quadratic
25	62.6%	82.6%	18.2%	27.1%	9.0%	11.2%
50	88.9%	100.0%	23.9%	35.7%	11.5%	13.2%
100	100.0%	100.0%	42.0%	57.0%	16.9%	18.1%
200	100.0%	100.0%	92.7%	87.6%	27.3%	24.0%
	subcritical $p_0 = 0.75$ $r(t) = \frac{1}{2}\{1 + \cos(\frac{t}{12})\}$		critical $p_0 = 0.5$ $r(t) = \frac{2}{1+(t-80)\vee 0}$		supercritical $p_0 = 0.45$ $r(t) = \frac{1}{2}e^{0.015t}$	
sample size	linear	quadratic	linear	quadratic	linear	quadratic
25	8.8%	78.6%	41.2%	37.1%	84.0%	78.5%
50	8.5%	99.7%	64.3%	57.8%	98.6%	96.9%
100	11.2%	100.0%	90.3%	84.9%	100.0%	100.0%
200	18.1%	100.0%	99.8%	99.0%	100.0%	100.0%

When the immigration rate was time-homogeneous (see top rows of Tables 1 and 2), the level of the test approached the nominal level of 5% as n increased, except when the process was supercritical where the level increased slightly when increasing the sample size from $n = 100$ to $n = 200$. The increase was possibly due to n being too large compared to t_1, violating Assumption 2. The level of the test was closer to the nominal ones when log-transforming the coefficients of variation (that is, when using $L(u) = \log u$), and this was true whether the process was sub-, super- or critical. The test also achieved better levels at small

sample sizes when time (t_j) was included only linearly in the vector of predictors (x_j) compared to when it was included both as a linear and a quadratic term (t_j^2). A possible strategy to reduce the rate of type-1 errors is to use nominal significance levels smaller than the traditional 5% level.

The power of the test increased with n. It was generally highest when the process was sub-critical or critical ($p_0 \leq 0.5$). With supercritical processes ($p_0 > 0.5$), larger sample sizes were required to achieve similar power because the contribution of the immigration to the dynamics of the population is eventually overwhelmed by that of the embedded Bellman-Harris process; that is, the cells that are present in the population eventually contribute substantially more to its dynamics than the immigration process, unless the immigration rate increases exponentially fast and has an exponent that is greater than the Malthusian parameter.

The choice of x_j (linear vs. quadratic) affected slightly the power of the test, except when the immigration rate oscillated over time (bottom of first column, $r(t) = \frac{1}{2}\{1+\cos(\frac{t}{12})\}$), where the test achieved limited power when constructed using $x_j = (1, t_j)^{\mathrm{T}}$. With samples of size 100, and when log-transforming the coefficients of variation (Table 1), it rejected H_0 in only 9.5% of the data sets. By comparison, with $x_j = (1, t_j, t_j^2)^{\mathrm{T}}$, the test regained power because W was built using a more flexible regression function, and rejected H_0 for 100% of the data sets.

We also observed that the test was slightly but consistently more powerful when constructed with log-transformed coefficients of variation ($L(u) = \log u$) than with untransformed coefficients of variation ($L(u) = u$), and this despite exhibiting lower type-1 error rates.

6. An application to the progression of leukemia

We conducted an experiment to study tumor growth in mice inoculated with leukemia stem cells. Eighteen mice were randomized into 2 groups of 9 mice each. In the first group, blood samples were collected every 24 hours from day 5 to day 10 post-inoculation; in the second group, samples were collected every 24 hours from day 5.5 to day 9.5. We quantified the number of leukemia blast cells and of normal (non-leukemic) cells using flow cytometry. Figure 3 shows the number of leukemic cells (panels A) and the number of normal cells (panels B) on a log-scale. The sample coefficients of variation are plotted against time in panels C and D.

As tumor burden increases, the number of normal cells in the blood changes over time primarily through immigration of cells from the bone marrow and through cell death. Leukemia cells change in number via immigration of cells from other tissues of the body (e.g., the bone marrow) or from differentiation of stem/precursor cells that are already in the blood. These cells also undergo self-renewing division. The impact of apoptosis (cell death) on cancer cells is likely limited, but these cells may exit the blood stream by migrating into other tissues and organs of the body. Thus, the pool of normal cells could be described

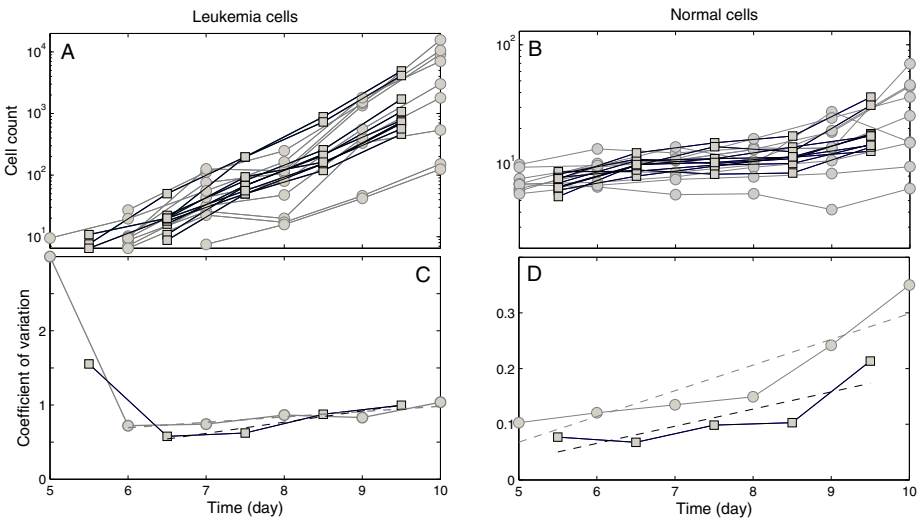

FIG 3. *Cell counts (log-scale; top panels) and sample coefficients of variation (bottom panels) for leukemia blast cells (left panels) and for normal cells (right panels) in two groups of mice (circles: group 1; squares: group 2) plotted against time.*

by a subcritical age-dependent branching process with immigration, whereas a supercritical process with immigration would be more appropriate to model the kinetics of leukemia cells.

The pool of normal cells in the blood expanded over time, and the increase in cell count accelerated around days 8–9. The number of leukemia cells increased exponentially, and there is no clear evidence that their immigration rate might have changed over time. The coefficients of variation for the number of leukemia cells were much higher on day 5 and on day 5.5 than in subsequent days, a possible consequence of the fact that some cell counts were too small to be reliably quantified at these time points. These values were excluded from the analyses. For both cell types, the coefficients of variation increased over time starting from day 6. The trend was similar for the two groups of mice, and suggested that the immigration rate was time-dependent. Based on Theorem 3.2, the shape of the coefficient of variation as observed for normal cells could be consistent with an immigration rate that decreases with time; for example, the immigration rate could have the form $r(t) = rt^{\theta}$, for some exponent $\theta \in (-1, 0)$. The methodology presented in this paper does not permit confirming this observation, however.

To assess the assumption that the immigration rate remained constant over time, we constructed our test using the coefficients of variation ($L(u) = u$) and their log-transformed values ($L(u) = \log u$), and considered two sets of linear predictors:

$$x_j = [1, g_j - 1, t_j, (g_j - 1)t_j]^{\mathrm{T}}$$

TABLE 3
P-values resulting from the analysis of the leukemia data

	$L(u) = u$		$L(u) = \log u$	
	linear	quadratic	linear	quadratic
normal cells	5.9×10^{-7}	7.8×10^{-6}	1.6×10^{-9}	1.7×10^{-14}
leukemia cells	0.051	0.054	0.13	0.027

and

$$x_j = [1, g_j - 1, t_j, (g_j - 1)t_j, t_j^2]^{\mathrm{T}},$$

where $g_j = 1$ or 2 in accordance with group membership (i.e., observed from day 5 or from day 5.5). In the first set of predictors (referred to as linear), t_j is entered linearly in the vector x_j, and we compared the value of our test statistic to a chi-squared distribution with two degrees of freedom: one coming from t_j and one from the interaction between t_j and g_j. In the second set of predictors (referred to as quadratic), t_j is entered in x_j both as a linear and as a quadratic term, and we compared the value of our test statistic to a chi-squared distribution with three degrees of freedom because three of the predictors were associated with t_j. We note that group membership (g_j) is included in x_j to allow for potential differences between the coefficients of variation computed in the first and in the second group of mice.

The p-values for normal cells and for leukemia cells obtained with these tests are presented in Table 3. They were all highly significant for normal cells, suggesting that immigration of these cells into the blood was likely time-dependent. Depending on the method used to construct the test statistic, the p-values of the coefficient of variation for leukemia cells were either non-significant but trending, or barely significant. Thus, immigration of leukemia cells was also possibly time-dependent. Immigration of leukemia cells could slow down as a result of cells exiting the mitotic cycle to undergo resting by entering the G0 phase. We performed additional experiments (see Figure 4) in which we measured the percentage of cells in the G0-G1 phase and in the S phase. We found that the former decreased while the latter increased over time. These results seemed to confirm our previous conclusions, which may have clinical implications. For example, it has been suggested that patient's survival may be prolonged by employing therapies designed to maintain the population of tumor cells at acceptable levels rather than attempting to eradicate all cancer cells, a strategy which could result in extensive damage to normal tissues. Such an approach would be viable if cancer cells were adapting their dynamics to their micro-environment, which is what our data suggests.

7. Conclusion

We have proposed a test to assess the assumption that the rate of immigration of an age-dependent branching process with immigration is time-dependent. These models find many applications in biology.

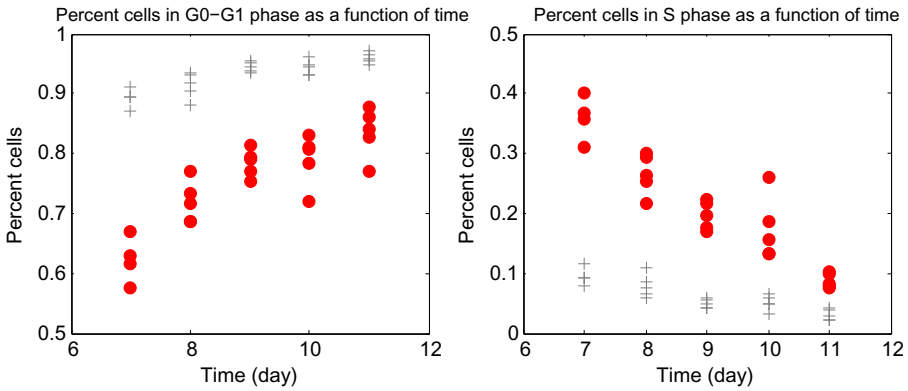

FIG 4. *Percent normal cells (+) and leukemia cells (o) in the G0-G1 phase (left panel) and in the S phase (right panel) plotted against time.*

Although the construction of our test hinges on the theory of branching processes, its implementation does not require any such process to be fitted. Furthermore, the distribution function of the lifespan and the shape of the immigration rate (if time-dependent) need not be specified. Since the asymptotic behavior of the coefficient of variation holds under fairly mild regularity conditions, this feature endows the test with robustness properties. Practically speaking, only required is fitting a linear model and estimating a variance-covariance matrix using (e.g.) a bootstrap procedure. It requires that multiple independent runs of the process be observed so coefficients of variation can be estimated, which is the case in many biological experiments.

The test exhibited good performances in simulation studies. As previously discussed, its power generally decreases when the embedded Bellman-Harris process is super-critical. We argued that this arises from the fact that immigration has less impact on the dynamics of the process than in the sub- and critical cases. This limitation is inherent to the property of super-critical processes. Thus, we do not expect it to be unique to the proposed test and it is likely that most, if not all, tests would suffer from this limitation under the same setting. Nonetheless, we found, in simulation studies, that the test exhibited reasonable power in situations similar those that may be encountered in practice. Another limitation of the proposed test is that it is constructed using the asymptotic behavior of the coefficient of variation, and t_1 has to be large enough so $C_v(t_j)$, $j = 1, \ldots, m$, are close to c_0, should H_0 be true. Exact derivation in the Markov case and our simulations (non-Markov case) suggested that convergence occurs quickly.

This test not only offers insights into cell kinetics, but provides also an important tool in model construction. For example, the analysis presented in Section 6 indicated that the influx of normal (and may be leukemic) cells into the blood stream should be modeled using a time-dependent immigration rate. Estimators for this class of processes are yet to be constructed before we can validate our conclusions. This will be done in future work.

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Appendix A: Asymptotic behavior of the mean and variance

We recall the following result (e.g, see [18]):

Theorem A.1 (Moments when immigration is time-homogeneous). *Assume that* $r(\cdot) \equiv r$.

(i) If $\alpha < 0$, *then* $M(t) \to r\gamma\overline{A}$, *and* $V(t) \to r[\gamma(\overline{B} + \overline{A}) + \gamma_2\overline{A^2}]$.
(ii) If $\alpha = 0$, *then* $M(t) = r\gamma t$, *and* $V(t) \sim \gamma r K_{B2} t^2/2$.
(iii) If $\alpha > 0$, *then* $M(t) \sim r\gamma K_A e^{\alpha t}/\alpha$, *and* $V(t) \sim r(\gamma K_{B3} + \gamma_2 K_A^2)e^{2\alpha t}/2\alpha$.

When the immigration rate changes over time, it follows from eqn. (3.5) that $M(t) = \gamma R(t)$ when $\alpha = 0$. When $\alpha \neq 0$, the asymptotic behavior of $M(t)$ is more complex. It is given in Theorem 3.2 for three important classes of immigration rates. Define the quantities: $\overline{E}_1(\alpha) = \int_0^\infty e^{-\alpha x}(1+x)^{-1}dx$, and $\widehat{A}_\rho = \int_0^\infty e^{-\rho u}A(x)dx$. Notice that $\overline{E}_1(\alpha) < \infty$ if $\alpha > 0$, and $\widehat{A}_\rho < \infty$ if $\rho > \alpha$.

Theorem A.2 (Expectation with time-inhomogeneous immigration).

Case 1. If $r(t) = r/(1+t)$, *then* $M(t) \sim \gamma\overline{A}r/(1+t)$ *if* $\alpha < 0$, *and* $M(t) \sim \gamma r\overline{E}_1(\alpha)A(t)$ *if* $\alpha > 0$.
Case 2. If $r(t) = rt^\theta$, $\theta > -1$, *then* $M(t) \sim \gamma\overline{A}r(t)$ *if* $\alpha < 0$, *and* $M(t) \sim \gamma r\Gamma(\theta+1)A(t)/\alpha^{\theta+1}$ *if* $\alpha > 0$.
Case 3. If $r(t) = re^{\rho t}$, $\rho > 0$, *then* $M(t) \sim \gamma\widehat{A}_\rho r(t)$ *if* $\rho > \alpha$, $M(t) \sim \gamma r A(t)/(\alpha - \rho)$ *if* $\rho < \alpha$, *and* $M(t) \sim \gamma r t A(t)$ *if* $\rho = \alpha$.

Theorem A.3 (Variance when immigration is time-nonhomogeneous).

Case 1. If $r(t) = r/(1+t)$, *then* $V(t) \sim [\gamma(\overline{B} + \overline{A}) + \gamma_2\overline{A^2}]r(t)$ *if* $\alpha < 0$, *where* $\overline{A^2} = \int_0^\infty A^2(x)dx < \infty$; $V(t) \sim \gamma r K_{B2} t \log(1+t)$ *if* $\alpha = 0$; $V(t) \sim r\overline{E}_1(\alpha)(\gamma K_{B3} + \gamma_2 K_A^2)e^{2\alpha t}$ *if* $\alpha > 0$.
Case 2. If $r(t) = rt^\theta$, $\theta > -1$, *then* $V(t) \sim [\gamma(\overline{B} + \overline{A}) + \gamma_2\overline{A^2}]r(t)$ *if* $\alpha < 0$; $V(t) \sim \gamma r K_{B2} t^{\theta+2}/(\theta+1)(\theta+2)$ *if* $\alpha = 0$; $V(t) \sim r\Gamma(\theta+1)(\gamma K_{B3} + \gamma_2 K_A^2)e^{2\alpha t}/(2\alpha)^{\theta+1}$ *if* $\alpha > 0$.
Case 3. If $r(t) = re^{\rho t}$, $\rho > 0$, *then* $V(t) \sim [\gamma(\widehat{A}_\rho + \widehat{B}_\rho) + \gamma_2\widehat{A^2_\rho}]r(t)$ *if* $\rho > 2\alpha$ *where* $\widehat{A}_\rho = \int_0^\infty e^{-\rho u}A(x)dx < \infty$, $\widehat{B}_\rho = \int_0^\infty e^{-\rho u}B(x)dx < \infty$, $\widehat{A^2_\rho} = \int_0^\infty e^{-\rho u}A^2(x)dx < \infty$; $V(t) \sim r(\gamma K_{B3} + \gamma_2 K_A^2)te^{2\alpha t}$ *if* $\rho = 2\alpha$; $V(t) \sim r(\gamma K_{B3} + \gamma_2 K_A^2)e^{2\alpha t}/(2\alpha - \rho)$ *if* $\rho < 2\alpha$.

Appendix B: Proof of Theorem A.2

It follows from eqn. (3.5) that $M(t)$ can be decomposed as $M(t) = \gamma\{J_1(t) + J_2(t)\}$, where $J_1(t) = \int_0^{\epsilon t} A(t-u)r(u)du$ and $J_2(t) = \int_{\epsilon t}^t A(t-u)r(u)du$, for

some constant $\epsilon \in (0,1)$.

Case 1. If $\alpha < 0$ then

$$r(t)\int_0^{t(1-\epsilon)} A(u)du \le J_2(t) \le r(t\epsilon)\int_0^{t(1-\epsilon)} A(u)du,$$

and we deduce that $J_2(t) \sim \overline{A}r(t)$. Next, for large enough t, there exists $\Delta > 0$ such that

$$J_1(t) \le (K_A + \Delta)A(t(1-\epsilon))\log(1+\epsilon t) = o(r(t)),$$

and we deduce that $M(t) \sim \gamma\overline{A}r(t)$.

Assume now that $\alpha > 0$. Since $\widetilde{A}(t) = e^{-\alpha t}A(t)/K_A \to 1$, we deduce that

$$M(t) = \gamma K_A e^{\alpha t}\int_0^t \widetilde{A}(t-u)e^{-\alpha u}r(u)du = \gamma K_A e^{\alpha t}\{I_1(t) + I_2(t)\},$$

where $I_1(t) = \int_0^{\epsilon t} \widetilde{A}(t-u)e^{-\alpha u}r(u)du$ and $I_2(t) = \int_{\epsilon t}^t \widetilde{A}(t-u)e^{-\alpha u}r(u)du$ for any $\epsilon \in (0,1)$. For large enough t, there exists $\delta \in (0,1)$ such that

$$(1-\delta)\int_0^{\epsilon t} e^{-\alpha u}r(u)du \le I_1(t) \le (1+\delta)\int_0^{\epsilon t} e^{-\alpha u}r(u)du,$$

from which we deduce that $I_1(t) \to r\overline{E}_1(\alpha)$. There exists also a constant $K > 0$ such that

$$I_2(t) \le K\int_{\epsilon t}^t e^{-\alpha u}(1+u)du \to 0.$$

Hence $M(t) \sim \gamma r\overline{E}_1(\alpha)A(t)$.

Case 2. Assume that $\alpha > 0$. Then for large enough t, there exists $\delta \in (0,1)$, such that

$$(1-\delta)r\int_0^{\epsilon t} e^{-\alpha u}u^\theta du \le I_1(t) \le (1+\delta)r\int_0^{\epsilon t} e^{-\alpha u}u^\theta du,$$

which implies that $I_1(t) \to r\Gamma(\theta+1)/\alpha^{\theta+1}$. Similarly to the previous case, we have that

$$I_2(t) \le Kr\int_{\epsilon t}^t e^{-\alpha u}u^\theta du \to 0,$$

which completes the proof in this case.

Assume next that $\alpha < 0$. Then

$$J_1(t) \le r\epsilon^\theta t^\theta \int_{t(1-\epsilon)}^t A(x)dx = o(r(t)).$$

Similarly to Case 1, we have, for $\theta \ge 0$, that

$$r(\epsilon t)\int_0^{t(1-\epsilon)} A(u)du \le J_2(t) \le r(t)\int_0^{t(1-\epsilon)} A(u)du,$$

and, for $\theta \in (-1, 0)$,

$$r(t)\int_0^{t(1-\epsilon)} A(u)du \le J_2(t) \le r(\epsilon t)\int_0^{t(1-\epsilon)} A(u)du,$$

from which we deduce, in both subcases, that $J_2(t) \sim \overline{A}r(t)$. Therefore $M(t) \sim \gamma\overline{A}r(t)$.

Case 3. Notice that

$$M(t) = \gamma r e^{\rho t}\int_0^t e^{-\rho u}A(u)du.$$

The proof follows directly from this expression when $\rho > \alpha$. When $0 < \rho \le \alpha$, the proof results from the fact that, for any $T \in (0, t)$,

$$M(t) = \gamma r e^{\rho t}\left\{\int_0^T e^{-\rho u}A(u)du + \int_T^t e^{-\rho u}A(u)du\right\} = \gamma r e^{\rho t}\{L_1(T) + L_2(t)\},$$

and, for large (but fixed) T, $L_1(T)$ is finite whereas, as $t \to \infty$, $L_2(t) \sim K_A e^{(\alpha-\rho)t}/(\alpha - \rho)$ when $\rho < \alpha$ and $L_2(t) \sim K_A t$ when $\rho = \alpha$.

Appendix C: Proof of Theorem A.3

When $\alpha \neq 0$, the proofs are similar to those of Theorem A.2 and they are omitted. They are only provided for Case 1 when $\alpha = 0$ (the proofs are similar in Cases 2 and 3).

Case 1. If $\alpha = 0$, then $B(t) = K_{B2}t + o(t)$, and algebraic calculations show that

$$\begin{aligned}\int_0^t r(t-u)udu &= r[t\log(1+t) - t + \log t] \\ &\sim rt\log(1+t).\end{aligned}$$

Moreover $\int_0^t r(t-u)o(u)du = I_1(t) + I_2(t)$, where, for any $\varepsilon \in (0, 1)$, $I_1(t) = r\int_0^{\varepsilon t} o(u)(1+t-u)^{-1}du$ and $I_2(t) = r\int_{\varepsilon t}^t o(u)(1+t-u)^{-1}du$. It can be shown that

$$I_1(t) \le r[1 + t(1-\varepsilon)]^{-1}\int_0^{\varepsilon t} o(u)du = r[1 + t(1-\varepsilon)]^{-1}o(t^2) = o(t).$$

For every $\delta > 0$, we can choose εt large enough such that $o(x) \le \delta x$ for $x \ge \varepsilon t$. Therefore

$$\begin{aligned}I_2(t) &= r\int_{\varepsilon t}^t o(u)(1+t-u)^{-1}du \\ &\le r\delta\int_0^{\varepsilon t} u(1+t-u)^{-1}du \\ &= r\delta\{t\log[1+t(1-\varepsilon)] - t(1-\varepsilon) + \log[1+t(1-\varepsilon)]\},\end{aligned}$$

from which we deduce that $I_2(t) = o(t\log[1+t])$. Hence $\int_0^t r(t-u)B(u)du \sim rK_{B2}t\log(1+t)$. Since $M(t) = \gamma\log(1+t)$, we finally deduce that $V(t) \sim \gamma r K_{B2}t\log(1+t)$.

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