Short Telomeres and a T-Cell Shortfall in COVID-19: The Aging Effect

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Abstract

Transient lymphopenia is a common feature of acute viral respiratory infections\(^1\). The drastic and prolonged lymphopenia of COVID-19, however, is distinctive and largely stems from falling counts of T cells\(^2^-^5\). This T-cell lymphopenia may contribute to the inordinate rise in COVID-19 mortality with older age\(^6\), because naïve T-cell clonal expansion is telomere length (TL)-dependent\(^7\) and TL of hematopoietic cells shortens with age\(^8\). Here we present a biologically plausible model that links naïve T-cell clonal expansion capacity and age-dependent hematopoietic cell TL (HCTL) shortening to explain the T-cell shortfall and the high COVID-19 mortality in older adults. The model shows that an individual with average HCTL at age twenty years maintains maximal T-cell clonal expansion capacity until the 6\(^{th}\) decade of life when this capacity plummets by more than 90% over the next eight years. The collapse coincides with the steep increase in COVID-19 mortality with age. As young adults tend to maintain their relative HCTL over their life course\(^9\), individuals with above and below average HCTL respectively experience the drop in maximal T-cell clonal expansion capacity at older and younger ages. HCTL metrics may thus explain the vulnerability of older adults to COVID-19 and predict the capacity for T-cell clonal expansion following vaccination against the virus.
The slow pace of global vaccination and the rapid emergence of SARS-CoV-2 variants suggest recurrent waves of COVID-19 in coming years\(^\text{10}\). Therefore, understanding why deaths from COVID-19 are highly concentrated among older adults is essential for global health, in particular for countries with a demography skewed towards older age. Declining immunity with advancing age is a general explanation\(^\text{6}\) but the increased mortality from COVID-19 in older adults exceeds that for most viral illnesses and it thus requires specific explanations. One of these might be diminished ability with age to offset the development of and recovery from severe T-cell lymphopenia that often complicates COVID-19\(^\text{2-5}\).

Much about the primary etiology of COVID-19 T-cell lymphopenia remains unknown, but regardless of its causes, offsetting the decline in T-cell count during SARS-CoV-2 infection demands fast and massive T-cell clonal expansion, which is telomere length (TL)-dependent\(^\text{7}\). This might explain recent studies that associate short hematopoietic cell TL (HCTL) with low lymphocyte count\(^\text{11,12}\) and severe disease\(^\text{13-15}\) in COVID-19 patients.

The T-cell blood pool size depends on a balance between T-cell depletion, due to senescence/death and sequestration out of the circulation, and T-cell repletion through T-cell proliferation. In absence of an acute infection, the T cell turnover is slow because of the relatively long biological half-lives of naïve T cells and memory T cells, i.e., \(~5\) years and \(~5\) months, respectively\(^\text{16}\). In the context of COVID-19 lymphopenia, however, diminished T-cell proliferation in older adults and other individuals with short HCTL could result in a shortfall between T-cell depletion and repletion\(^\text{12}\). Moreover, the clearance of SARS-CoV-2 requires clonal expansion and differentiation of naïve T cells into SARS-CoV-2 antigen-specific memory T cells\(^\text{2,3,5}\). Short naïve T-cell telomeres might thus limit adaptive immunity against the
virus even without a clinical manifestation of T-cell lymphopenia. Making reasonable assumptions, we therefore modelled the relationships of TL-dependent T-cell clonal expansion with age.

### The Model

**Note: Abbreviations, symbols and units used in the mathematical development and testing the model are presented in Box 1.**

The following assumptions on T-cell replication and clone size (CS) drive our model (Fig. 1): (i) T-cell TL-dependent cessation of replication is defined by a “telomeric brink” (TL\(_B\)) that stops replication at 5 kilobases\(^8\). (ii) TL of a naïve T cell at age 20 years (TL\(_{20}\)) progressively shortens at a rate g of 0.03 kb/year\(^17,18\) until it reaches the TL\(_B\). (iii) In exponential growth, i.e., 1→ 2→ 4→ 8→16, etc., a single naïve T cell can generate a maximal CS (MCS) of ~ 10\(^6\) (one million) memory T cells through ~ 20 replications (N\(_{\text{max}}\)); this value was estimated based on the ~ 1.4 kb TL difference between circulating naïve and memory T cells and the ~ 0.07 kb telomere shortening per replication of cultured T cells\(^19\). (iv) We denote the maximal TL shortening due to clonal expansion as Δ\(_{\text{max}}\) and the TL shortening per replication as r. (v) Due to age-dependent TL shortening, a naïve T-cell TL reaches the “telomeric onset” (TL\(_O\) = 6.4 kb) at “age of onset” (X\(_O\)). (vi) Until X\(_O\), a naïve T cell can generate MCS. After X\(_O\), a naïve T cell can generate only a limited clonal size (LCS < MCS), as the TL of the clonal cells converges to the TL\(_B\). (vii) Most memory T cells are formed in response to new antigens

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**Box 1. Abbreviations, meanings, units and values**

| Abbreviation | Meaning | Units |
|--------------|---------|-------|
| TL\(_{20}\)  | TL at age at 20 years (kb) | |
| TL\(_x\)     | TL at x years older than 20 years (kb) | |
| TL\(_B\)     | Telomeric brink, stopping cell replication | Set at 5 kb in the model |
| TL\(_O\)     | TL at onset of clone size limitation | Set at 6.4 kb in the model |
| X\(_O\)      | Age in years when TL\(_O\) is reached | |
| CS           | Clone Size | |
| MCS          | Maximal Clone Size (~ one million T cells, denoted 10\(^6\)) | |
| LCS          | Limited Clone Size (< 10\(^6\) T cells) | |
| Δ\(_{\text{max}}\) | TL shortening required for achieving MCS (~ 1.4 kb) | |
| g            | TL shortening rate with age (~ 0.03 kb/year) | |
| r            | TL shortening rate per T-cell replication (~ 0.07 kb) | |
| N\(_{\text{max}}\) | N of T-cell replications to achieve MCS (before TL\(_O\), X\(_O\)) | 20 replications to produce MCS of ~ 10\(^6\) T cells |
| N            | Number of T-cell replications to achieve LCS (after TL\(_O\), X\(_O\)) | |

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during childhood and early adulthood\textsuperscript{20}, when HCTL is comparatively long\textsuperscript{21,22}, enabling the achievement of MCS.

We define CS by the number (N) of T-cell replications producing \( CS = 2^{N} \). As a clone expands, TL of its T cells progressively shortens, i.e., \( \Delta = rN \), where \( r \) is the telomere shortening due to T-cell replication.

Prior to \( X_{O} \), the maximum number of T-cell replications in clonal expansion is \( N_{\text{max}} = \Delta_{\text{max}} \div r = 20 \). After \( X_{O} \), the number of T-cell replications in clonal expansion is \( N = (\text{TL}_X - \text{TL}_B) \div r \), where \( X \) designates age and the corresponding age-dependent TL of a naïve T cell is \( \text{TL}_X = \text{TL}_B - g(X - 20) \), where \( g \) is the TL shortening in the naïve T cell each year. The resulting \( X_{O} \) is the number of years it takes for a naïve T cell to reach TL\(_O\). These measures are defined by

\[
X_{O} = 20 + (\text{TL}_B - \text{TL}_B - \Delta_{\text{max}}) \div g
\]

\[
\text{TL}_O = \text{TL}_B + \Delta_{\text{max}}
\] (1)

The clone size depends on age relative to the \( X_{O} \) as follows

\( X \leq X_{O} \quad \text{MCS} = 2^{N_{\text{max}}} = 2^{\Delta_{\text{max}} \div r} \)

\( X > X_{O} \quad \text{LCS} = 2^{N} = 2^{(\text{TL}_B - \text{TL}_B - g(X - 20)) \div r} \) (2)

The following considerations are relevant for appraising the model’s parameters and assumptions: First, while the MCS is based on the \textit{in vivo} TL difference between naïve and memory T cells, the data on telomere shortening per T-cell replication are from cultured cells\textsuperscript{19}. A similar approach (based on data from circulating hematopoietic cells and telomere shortening in cultured cells) was previously used to generate consistent information on hematopoietic cell replicative kinetics\textsuperscript{23,24}. Second, the model’s TL parameters are derived from a large population-based study\textsuperscript{8} that measured HCTL by Southern blotting\textsuperscript{25}. Its telomere data are consistent with another large-scale study that used Flow-FISH to measure HCTL\textsuperscript{26}. Third, the model is based on age-dependent shortening of HCTL and not T-cell TL. As TL differences among leukocyte lineages within the individual are far smaller than the inter-individual HCTL variation\textsuperscript{27}, T-cell TL largely mirrors HCTL. Fourth, the TL signal for cessation of cell replication
originates from the shortest telomeres in the nucleus and not their mean TL. Using the Telomeres Shortest Length Assay (TeSLA), a method that tallies and measures the shortest telomeres, we recently showed that in patients with COVID-19 the shortest telomeres in peripheral blood mononuclear cells were associated with low lymphocyte counts. The principles that drive our model thus likely apply to the T cell’s shortest telomeres.

**Results**

Consider three individuals with average, long (one SD above the mean) and short (one SD below the mean) naïve T-cell TL (Fig. 2a): Their naïve T-cells display a slow pace of shortening at a rate of 0.030 kb/year (Fig. 2b). Up to X₀ of 50 years (TL₀ = 6.4 kb), the naïve T cells of the individual with the average TL₂₀ can attain the MCS of ~10⁶ T cells. Thereafter, however, the ability of these naïve T cells to clonally expand plummets from the MCS to 0.5 x 10⁶, 0.25 x 10⁶ and 0.125 x 10⁶ T cells at ages 53, 55 and 57 years, respectively (Fig. 2c). Next consider the individual with long T-cell TL (Fig. 2a). The ability of naïve T-cells of this individual to achieve MCS extends to the X₀ of 70 years (TL₀ = 6.4 kb) (Fig. 2c). This suggests that even among older adults, some individuals can develop MCS when infected by SARS-CoV-2. In contrast, naïve T cells of the individual with short T-cell TL are able to achieve MCS only until the X₀ of 30 years (TL₀ = 6.4 kb) (Fig. 2c), inferring that some young adults might generate a poor T-cell response to SARS-CoV-2 infection. A three-dimensional configuration of the effect of age on T-cell TL and CS dynamics is displayed in Fig. S1, Supplementary Information A.

Our model thus suggests that in the overwhelming majority of the general population, naïve T cells can achieve MCS before early adulthood. Later, post X₀, while age-dependent shortening of T-cell telomeres remains slow (Fig. 2b), it has a striking effect on clonal expansion of naïve T cells because the exponential nature of the process (Fig. 2c). As SARS-CoV-2 memory T cells play a greater role than neutralizing antibodies in recovering from the infection, such an effect could impede adaptive immunity.
and heighten the risk for severe COVID-19. Moreover, we assume that MCS of $\sim 10^6$ T cells applies not only for naïve T cells that clonally expand to produce memory T cells but also naïve T cells that clonally expand to produce naïve T cells. This means that regardless of the primary cause of COVID-19 T-cell lymphopenia, the T cell response will be compromised post $X_0$.

With aging, T cells of an increasing proportion of the population reach $X_0$ (Fig. 3a). After reaching this TL landmark, LCS contracts to less than 10% of MCS during the course of 8 years and to 1% over 16 years. This rapid LCS contraction means that at any age, the vast majority of individuals separate into two sub-populations; those with naïve T cells that can generate MCS and those with naïve T cells that can only generate LCS, which a few years after $X_0$ is a small fraction of MCS. While naïve T cells of individuals in their twenties can overwhelmingly generate MCS, naïve T cells of the majority of individuals in their seventies can at best generate clones that are typically less than one tenth of MCS (Fig. 3b).

What then might be the minimal TL-dependent T-cell CS that enables survival of an individual contracting COVID-19? The definitive answer can only come from telomere and T-cell data in a large population of COVID-19 patients. That said, we infer this CS based on Centers for Disease Control and Prevention (CDC) reports of the age-specific COVID-19 mortality rates for the United States and the population size by age in 2019. We computed an age-dependent hazards ratio of mortality relative to age 20 years (Hazards$_{20}$) from COVID-19 and from general causes other than COVID-19 (Methods). The Hazards$_{20}$ yields an exponential increase of COVID-19 mortality with age, and for comparison, we also display Hazards$_{20}$ for mortality from non-COVID-19 causes (Fig. 4a).

We assumed that, as a subpopulation, individuals who generate MCS experience no T-cell TL-related COVID-19 mortality. We therefore calculated the mean CS for the LCS subpopulation only, i.e. adults
older than $X_0$ (Fig. 3a). The TL-limited clonal expansion of individuals in the LCS subpopulation, we assumed, might contribute to their propensity to die from COVID-19, given the association between lymphopenia and COVID-19 mortality. Figure 4b shows that the mean LCS in adults older than $X_0$ decreases in a near linear manner with age. Plots of the mean LCS vs. Hazards ratios of COVID-19 mortality and non-COVID-19 mortality suggests a divergence between the two trajectories near the start of the 6th decade (Fig. 4c). The figure also displays the mean LCS at the age of 50 years, which corresponds to the age at which the size of LCS subpopulation is similar to that of the MCS subpopulation (Fig. 3b). Mean LCS at this age amounts to $\sim 0.13 \times 10^{-6}$ T cells.

The divergence of COVID-19 mortality from non-COVID-19 mortality when the mean LCS is about one tenth of MCS suggests the following: In the absence of COVID-19, $\sim 10\%$ MCS is generally sufficient to accommodate the low turnover of T-cells. This LCS, however, might contribute to mortality in the face of SARS-CoV-2, because the infection demands massive T-cell clonal expansion to offset the primary cause of the dropping naïve T-cell count, and to generate memory T-cells that clear the virus.

**Discussion**

With the exception of heritability, no other single factor so profoundly affects HCTL as does aging, explaining the key conclusion of our model. As illustrated in Figs. 2, 3, our model also applies to naïve T cells of a small subset of younger adults, whose HCTL is ranked at the lower range of the HCTL distribution. Specific groups might have diminished naïve T-cell clonal expansion in response to SARS-CoV-2 infection because of comparatively shorter HCTL. These include males, whose HCTL is shorter than in females from birth onwards. These groups also include persons with atherosclerotic cardiovascular disease, obese persons and smokers, whose HCTL is respectively shorter than that
in healthy, lean and non-smoking individuals. All these groups are at a higher risk of severe COVID-19 and death from the disease\textsuperscript{38-41}.

Humans have comparatively short telomeres relative to their long lifespan\textsuperscript{42}, and therefore our model may not apply to most terrestrial mammals, including laboratory animal models that are used for viral research. For instance, TL-mediated replicative aging is probably not consequential during the 2-3-year lifespan of mice, considering their long telomeres (mean TL > 30 kb) and robust telomerase activity in their somatic cells\textsuperscript{42-44}. In contrast, the average human TL at birth is only \textsim\ 9.5 kb\textsuperscript{21}. As telomerase activity is repressed in replicating human somatic cells\textsuperscript{42-44}, their short telomeres experience further age-dependent shortening after birth. Although naïve T cells have some telomerase activity\textsuperscript{7,19}, it is insufficient to prevent their age-dependent telomere shortening, and aging may thus undermine the T-cell clonal expansion in many humans.

After reaching $X_0$, our model predicts that naïve T cells will stall in generating through clonal expansion not only antigen-specific memory T cells but also new naïve T cells. Two converging lines of evidence support this idea: The numbers of naïve CD8 T cells decline with age\textsuperscript{45}. As a low count of naïve CD8 T cells contributes to severe COVID-19\textsuperscript{5}, individuals with short HCTL might thus be at a heightened risk for severe disease. Second, whereas lymphopenia is a major prognostic feature of COVID-19 in adults, when present, it is transient and of little prognostic value in children\textsuperscript{46-49}. The longer HCTL during early life potentially explain this clinical course.

Relatedly, the model shows that naïve T-cells with TL\textsubscript{20} of one SD below the mean are unable to achieve MCS as early as after the third decade of life. This unexpected finding suggests that (a) underestimation of naïve T-cell TL using population-based HCTL data, or (b) in response to pathogens, more
naïve T cells might be tapped for clonal expansion in young adults with short T-cell telomeres. Older adults may not have sufficient naïve T cells, particularly, naïve CD8 T cells, for this purpose.

We acknowledge the following limitations: Our model is deterministic with fixed rates and thresholds selected as best estimates from available literature. That said, analysis of the effects of assumptions on the LCS definition and uncertainty in the parameters \( r, g, \text{TL}_0 \) and \( \Delta_{\text{max}} \) indicate that the \( \text{TL}_0 \) and \( X_0 \) exhibit little error from the LCS assumption and parameter uncertainties. (Supplementary Information B2). The model draws on HCTL data from populations comprising principally whites of European ancestry in high-income countries. It should also be tested in populations of different ancestries and in low- and middle-income countries. The TL difference between naïve T cells and memory T cells likely reflects the clonal expansion in response to not only a single encounter but multiple encounters with a given antigen and its cross-reactive antigens. Thus, the MCS and LCS definitions in absolute T-cell numbers might be off the mark. Of note, however, the MCS and LCS can be expressed in the model in relative units of MCS (i.e., 0.5 MCS, 0.25 MCS, etc.) rather than absolute units (i.e., \( 0.5 \times 10^6 \), \( 0.25 \times 10^6 \) T cells, etc.), yielding identical results to those based on absolute T-cell numbers. Therefore, the principles of our model are likely to hold notwithstanding the above limitations.

In conclusion, the age-dependent TL effect on naïve T-cell clonal expansion likely influences the outcome of SARS-CoV-2 infection. The insight generated by our model might set the stage for measurement of TL parameters not only in older adults but also the general adult population, helping to identify individuals vulnerable to severe COVID-19 because of short T-cell telomeres. These individuals might also be less likely to develop a lasting T-cell-mediated immunity in response to anti-SARS-CoV-2 vaccines. Finally, the ramifications of these conclusions go beyond the influence of TL on T-cell response to SARS Cov-2 infection and vaccination against the virus. They suggest that TL might be a limiting factor.
in immunotherapies whose efficacies depend on clonally expanding (in vivo and in vitro) transplanted hematopoietic cells, chimeric antigen receptor T cells, and tumor-infiltrating lymphocytes.

**Methods**

The relationships between the age-dependent T-cell TL density and the relative proportion of CS in a population (Fig. 3) were derived from the distribution of HCTL at age twenty, extrapolated from age-specific density plots of HCTL. In Fig. 3a, the T-cell TL distribution for age 20 was derived from 1,000,000 random generations from a normal distribution with a mean = 7.3 kb and SD = 0.6 kb.

The combined male and female hazards ratios relative to age 20 in Fig. 4a were calculated from age-specific COVID-19-linked mortalities and total non-COVID-19 linked mortalities normalized by the age-specific US population. The, Hazards$_{20} = \left( \frac{mortality_{age}}{population_{age}} \right) \left( \frac{mortality_{20}}{population_{20}} \right)$, are based on the CDC records of 494,234 provisional COVID-19 deaths and 3,845,819 total deaths between January 1, 2020 through March 8, 2021 and the 2019 US Census. Non-COVID-19 mortalities were estimated by subtracting the COVID-19 mortalities from the total mortalities for each age group.

The definition of the LCS subpopulation sets the MCS at which TL-dependent COVID-19 mortality can occur. The LCS subpopulation includes any CS < MCS. Correspondingly, other definitions, i.e., cutoff levels, for the category, e.g., LCS < 0.5 MCS or LCS < 0.15 MCS, result in different relationships of mean LCS with age and Hazards$_{20}$. Supplementary information B2 explores the effects of these alternative LCS cutoff levels on Figs. 3 and 4. Lower cutoff levels have minor effects on which histogram bars in Fig. 3 are assigned to the LCS and MCS subpopulations. For Fig. 4b, lower cutoff levels reduce the mean LCS at age 20 years but not at age 80 years. Consequently, the relation of mean LCS with age is nearly linear for all LCS cutoff levels. Lower cutoff levels reduce the range of the mean LCS scale, i.e., rescales the x axis (Fig. 4c). In essence, this rescaling does not change the relative shape of the Hazards$_{20}$ curves for both
COVID-19 and non-COVID-19 mortalities, but does change the LCS values at which the two curves diverge. This change does not impact, therefore, the model’s qualitative response with age.

Furthermore, the uncertainty in the absolute value of CS is mitigated by expressing LCS as a fraction of the MCS. Finally, the LCS is not used for developing Fig. 2.

Codes for generating the figures are provided in Supplementary Information C.

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Fig. 1 | Age-dependent T-cell telomere length (TL) before (▬) and after (▬) clonal expansion. Naïve T-cell clonal expansion shortens telomeres by $\Delta$, where $\Delta_{\text{max}}$ is T-cell telomere shortening resulting from expansion to form the maximal clonal size (MCS). The telomeric brink ($\text{TL}_{\text{B}}$) of 5 kb is TL that increases the risk of cessation of replication. $\text{TL}_{20}$ is TL at 20 years, $\text{TL}_{\text{O}}$ is telomeric onset, which indicates the shortest T-cell TL that enables attaining MCS. $X_{\text{O}}$ is age of onset of clonal expansion limitation.
Fig. 2 | Population distribution of T-cell TL at age 20 (TL\textsubscript{20}) (a), T-cell TL shortening with age (b), and age-dependent change in T-cell clone size (CS) (c). a displays the TL\textsubscript{20} distribution, showing mean TL = 7.3 kb (▬), long TL (mean + SD) = 7.9 kb (▬), and short TL (mean – SD) = 6.7 kb (▬). b displays age-dependent change in T-cell for mean, long and short TL\textsubscript{20}. Past the telomeric onset (TL\textsubscript{O} = 6.4 kb), TL is insufficient to produce MCS because a full clonal expansion drops TL below the telomeric brink (TL\textsubscript{B} = 5 kb). The TL\textsubscript{O} is reached at different ages of onset (X\textsubscript{O}), i.e., an older age for T-cells with long T-cell telomeres and younger with T-cells with short telomeres. The age-dependent T-cell TL shortening (0.030 kb/year) for T cells with mean, long and short telomeres at TL\textsubscript{20} is shown by the lines. c shows that the T-cell CS is partitioned by the X\textsubscript{O} into plateau and slope regions. T cells with mean, long or short TL\textsubscript{20} achieve MCS on the CS plateau, but their CS exponentially collapses (slope) once their TLs shorten below TL\textsubscript{O} of 6.4 kb and exceed X\textsubscript{O} (at different ages).
**Fig. 3** | Shifts by age in naïve T-cell TL distribution (a) and relative frequency (0 to 10) of T-cell clone size (CS) (b) in the population.  

**a** displays the shift in TL\textsubscript{0} distribution (Fig. 2a) resulting from age-dependent shortening of 0.030 kb/year. It depicts TL < TL\textsubscript{0} (6.4 kb) by blue bars and TL > TL\textsubscript{0} by red bars. **b** displays relative frequency of CS's generated by naïve T-cell clonal expansion corresponding to the categories of TL below or above TL\textsubscript{0}. It shows that maximal CS (MCS) of ~ 10\textsuperscript{6} cells occurs in individuals with naïve T-cell TL > TL\textsubscript{0}, while limited CS (LCS) occurs in those with naïve T-cell TL ≤ TL\textsubscript{0}. At age 20, naïve T cells of nine out of ten individuals can generate MCS. At age 70, naïve T cells of less than two out of ten individuals can generate MCS, and seven out of ten generate clone sizes that are less than 0.1 MCS. At age 50 the population is approximately equally divided between the MCS and LCS groups.
**Fig. 4** Steps linking mean limited clone size (LCS) to COVID-19 mortality and general mortality hazards ratios in the population. 

**a** displays data based on COVID-19 mortality (●) and non-COVID-19 mortality (★), and corresponding exponential fitted relationships for hazards ratios (▬ and ▬). 

**b** displays the relationship of mean LCS in units of $10^6$ cells with age, generated with equation 2, using the TL distribution of Fig. 2a. 

**c** displays the relationships of hazards ratios generated from COVID-19 mortality and non-COVID-19 mortality plotted against mean LCS obtained from **b**. The top of the panel also displays age. The divergence between the COVID-19 and non-COVID-19 mortalities occurs at mean LCS of ~ $0.13 \times 10^6$ T cells. At the corresponding age, 50 years, the population is about evenly divided into the LCS and MCS sub-populations (Fig. 3b). After this age, the majority of the population is in the LCS group, which is susceptible to COVID-19 mortality, whereas the MCS group is not.