

ESTIMATING THE NUMBER OF HIV-SPECIFIC T-CELLS IN HEALTHY DONORS USING HIGH-THROUGHPUT SEQUENCING PROFILES OF T-CELL RECEPTOR REPERTOIRES


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In-depth study of mechanisms of immune response to the human immunodeficiency virus (HIV) is critical for better understanding of how immunodeficiency develops in patients with HIV, as well as for designing effective immunotherapy strategies and vaccines against the virus. In this work we analyze sequencing profiles of T-cell receptor repertoires previously obtained from healthy donors (601 Americans and 65 Russians) to estimate the population frequency of HIV-specific naive T-cells. We demonstrate that frequencies of T-cells recognizing different HIV epitopes vary considerably across the population (F-statistic = 2007, $p < 10^{-100}$, ANOVA). Although the frequency of T-lymphocytes recognizing a particular epitope does not change significantly between the individuals, it still largely depends on the presence of certain HLA alleles ($p < 0.01$, post-hoc Tukey's test), cytomegalovirus infection (F = 61, $p = 7 \times 10^{-15}$, ANOVA), and age (Pearson correlation coefficient ranging from -0.53 to -0.14 in different groups).

Keywords: HIV, T-cell receptor, HLA, CMV, high-throughput sequencing

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ОЦЕНКА ЧИСЛА ВИЧ-СПЕЦИФИЧНЫХ Т-ЛИМФОЦИТОВ У ЗДОРОВЫХ ДОНОРОВ ПО ДАННЫМ ВЫСОКОПРОИЗВОДИТЕЛЬНОГО СЕКВЕНИРОВАНИЯ РЕПЕРТУАРОВ Т-КЛЕТОЧНЫХ РЕЦЕПТОРОВ

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Детальное изучение механизмов формирования иммунного ответа против вируса иммунодефицита человека (ВИЧ) необходимо для лучшего понимания процессов развития иммунодефицита у пациентов с ВИЧ-инфекцией, разработки эффективных методов ее иммунотерапии и новых вакцин против вируса. В исследовании нами были проанализированы данные ранее полученных результатов секвенирования репертуаров Т-клеточных рецепторов для большого числа здоровых индивидуумов (601 донор из американской популяции и 65 доноров из российской популяции) с целью определения частоты наивных Т-лимфоцитов, обладающих специфичностью к ВИЧ, в популяции. Мы обнаружили, что на уровне популяции имеет место значительная вариабельность между различным эпитопами ВИЧ в частотах специфичных Т-клеток (F-statistic = 2007, $p < 10^{-100}$, ANOVA). Несмотря на то, что частота Т-лимфоцитов, специфичных к одному и тому же эпитопу, была достаточно стабильной между индивидуумами, мы обнаружили, что она значительно зависит от таких факторов, как наличие у человека определенных аллелей HLA ($p < 0,01$, пост-хок тест Тьюки), наличие цитомегаловирусной инфекции (F = 61, $p = 7 \times 10^{-15}$, ANOVA), а также возраст (коэффициент корреляции Пирсона в диапазоне от $-0,53$ до $-0,14$ для разных групп).

Ключевые слова: ВИЧ, Т-клеточный рецептор, HLA, CMV, высокопроизводительное секвенирование

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The highly mutable human immunodeficiency virus (HIV) easily evades the immune system [1]. Still, there is hope for anti-HIV immunotherapy, considering the variety of immunogenic HIV epitopes [2] and protective human leukocyte antigen (HLA) alleles [3], as well as the phenomenon of the so-called elite controllers [4]. Although attempts to develop an HIV vaccine have not paid off yet, the accumulated evidence suggests that the T-cell therapy may potentially be as effective as the conventional antiretroviral treatment [5].

In this work we draw on the assumption that the proportion of antigen-specific T-cells occurring in the naive T-lymphocyte

population determines the magnitude of the immune response [6]. Knowing which HIV epitopes tend to be readily recognized by the immune system of a person who carries a particular set of HLA alleles will help to elucidate mechanisms of immune protection against HIV and finally make headway in the development of an HIV vaccine.

The ability of T-cells to recognize foreign antigens is encoded by alpha- and beta-chain genes of T-cell receptors (TCR). Their diversity is incredible: the number of unique sequences (variants) in a person's beta chain is estimated to be over 10^9 , while the total number of TCR variants generated in the thymus

gland of each member of the population is almost infinite [7]. Massively parallel sequencing of immune repertoires (RepSeq) has evolved to simultaneously produce millions of TCR reads per studied sample, e. g. of peripheral blood mononuclear cells [8]. Currently existing methods of T-cell sorting, especially those based on MHC multimer staining [9], yield a wealth of information about antigen-specific TCR. In this light, RepSeq can be conveniently used to analyze individual TCR repertoires. For example, data generated by RepSeq can be further annotated *in silico* and the number of epitope-specific T-cells can be estimated using the regularly updated VDJdb repository of TCR sequences with known antigen specificity [10].

That said, it is almost impossible to accurately quantify antigen-specific T-cells in the naive T-cell population using standard techniques, such as flow cytometry. Because the population of T-cells that recognize a particular epitope is often very small (< 1 %) [11], magnetic bead enrichment may be needed [12], which, unfortunately, can distort the results. In contrast, RepSeq reliably reports T-cells with frequencies as low as 0.001 % [13].

Having a large dataset of TCR sequences at our disposal obtained from 65 Russian and 601 American donors and another dataset of 1,688 TCR with known epitope specificity (see Methods), we have attempted to study the frequency of HIV-specific T-cells in the population. The following hypotheses have been tested:

- 1) frequencies of epitope-specific T-cells in the TCR repertoires of healthy individuals vary considerably depending on the epitope;
- 2) cytomegalovirus (CMV) infection in the individual affects the proportion of HIV-specific T-cells in his T-cell repertoire;
- 3) the number of HIV-specific T-cells depends on the presence of specific HLA alleles in the individual;
- 4) the number of HIV-specific T-cells depends on the individual's age and sex.

METHODS

We analyzed the datasets of sequenced TCR beta chains obtained by Emerson et al. [14] and Britanova et al. [15]. We

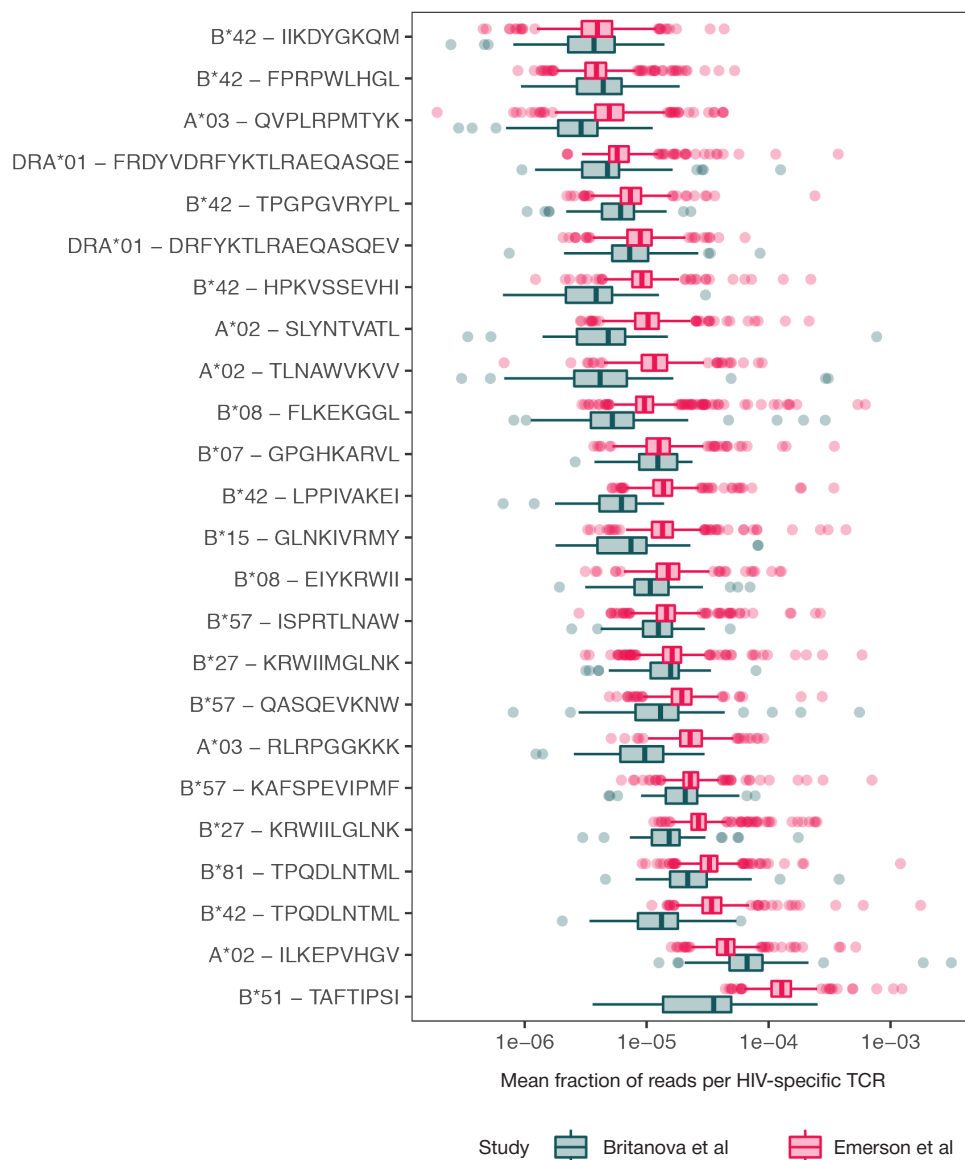


Fig. 1. Mean abundance of different HIV-specific T-cell receptors (TCR) in the T-cell repertoires sequenced by Emerson et al. [14] and Britanova et al. [15]. The X-axis represents distribution of an average-sized clonotypic population derived from an HIV-specific T-cell (fraction of all TCR beta chain reads) in the samples from [14] (n = 65) and [15] (n = 601). The Y-axis represents epitopes for which HIV-specific TCR sequences are known (according to VDJdb); data format: HLA allele : epitope sequence

did not use all of the sequenced data obtained by Emerson, selecting the TCR repertoires of only those donors whose HLA haplotype had been identified and CMV status was known — a total of 601 samples. We also filtered out umbilical cord blood TCR from Britanova et al. study's sample, saving for the analysis only the repertoires of 65 healthy adults. Data preprocessing and segment mapping for sequences borrowed from [15] were performed with MIGEC [16] and MiTCR [17] software tools. Segments from [14] were additionally mapped, V- and J-segment genes were identified and sequencing errors were corrected using MiXCR [18]. Data were cleaned of non-functional sequences containing stop-codons or frameshifts using VDJtools [19].

Annotation, i. e. prediction of HIV-specific TCR, was done using VDJtools/VDJdb-standalone [10]. VDJdb was searched for HIV-specific TCR; epitopes represented in the database by less than 10 TCR variants were excluded from the analysis. A RepSeq TCR was counted as specific to a particular epitope if the amino acid sequence of the epitope's hyper variable CDR3 region differed by no more than 1 substitution from the corresponding TCR sequence stored in VDJdb. This approach yields a substantially larger set of annotated TCR, with only a tiny percent of erroneous annotations, as shown in [10].

Statistical analysis was done with R. The following statistical algorithms were used: ANOVA, Tukey's post hoc test and correlation analysis. Values for the F-statistic, Spearman's rank correlation and Student's p are provided in the Results section.

RESULTS

Frequency estimates obtained by flow cytometry for HIV-specific TCR convincingly demonstrate that the proportion of specific T-cells in the naive (intact) repertoire varies considerably, differing by 1 or 2 orders of magnitude between the epitopes, while remaining fairly stable between different individuals [12]. Analysis of high-throughput TCR sequencing data conducted in the course of our study (Fig. 1) supports these observations: frequencies of HIV-specific TCR have been found to be highly epitope-dependent ($F = 2007$, $p < 10^{-100}$, ANOVA), which, however, bears no connection to the presenting HLA allele ($F = 0.03$, $p = 0.86$, ANOVA). Importantly, there is a significant discrepancy in the estimates for Emerson's and Britanova et al. study's datasets ($F = 1690$, $p < 10^{-100}$; average frequency of HIV-specific TCR is higher for Emerson et al. study's data), which can be explained by different structures of TCR libraries and techniques used for their preparation. Emerson et al. worked with DNA samples employing multiplex PCR, while Britanova et al. used RNA samples, 5'RACE and molecular barcoding [20]. Skipping the details, we will, however, emphasize that molecular barcoding ensures more accurate quantification of TCR in the sample [15].

In the study by Emerson et al. the donors were divided into two cohorts based on their serologic status, i. e. on the presence or absence of CMV infection. With sequencing data at our disposal, we seized this opportunity to evaluate

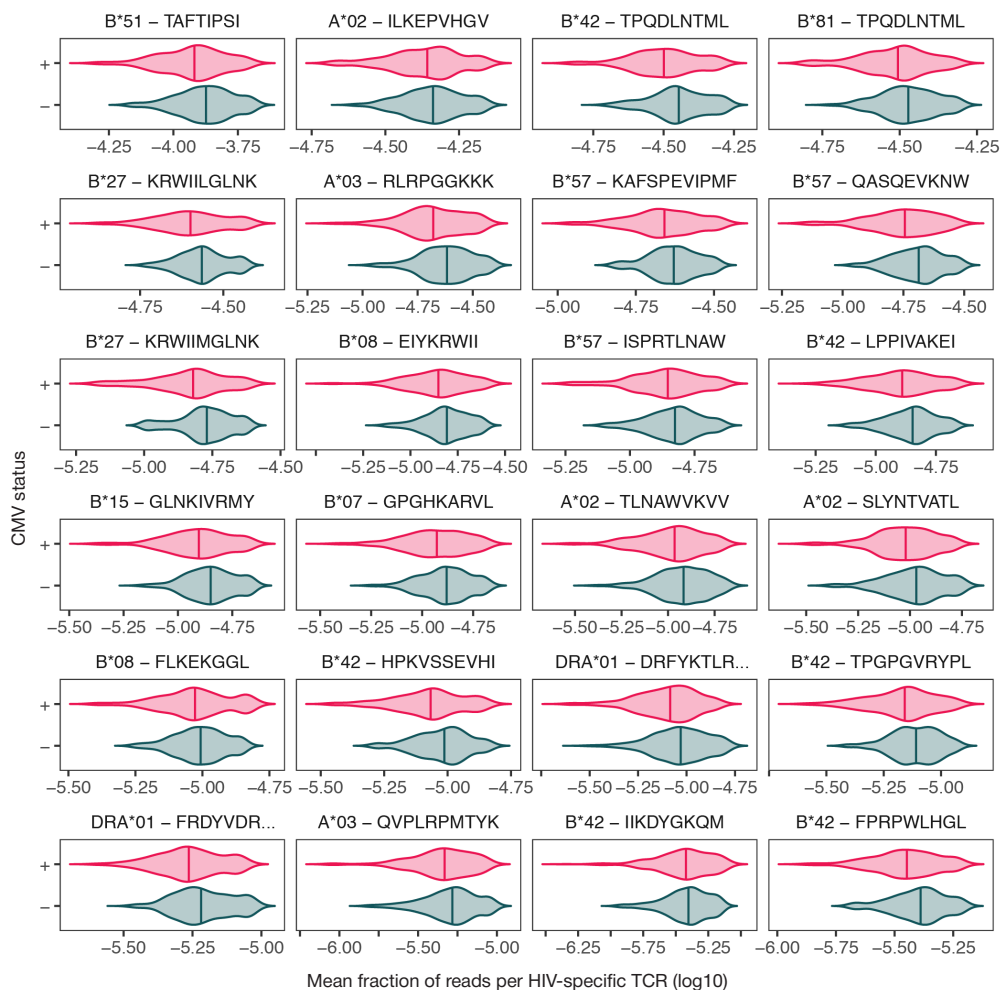


Fig. 2. Abundance of HIV-specific TCR in the sample of individuals with known cytomegalovirus status. Results for CMV-positive (+) and CMV-negative (-) individuals are provided separately for each epitope. Plotted along the X-axis is the common logarithm of an average-sized clonotypic population of an HIV-specific T-cell (fraction of total TCR beta chain reads). Data have been winsorized for graphical clarity: the figure shows samples with values ranging between the 5th and 95th percentiles

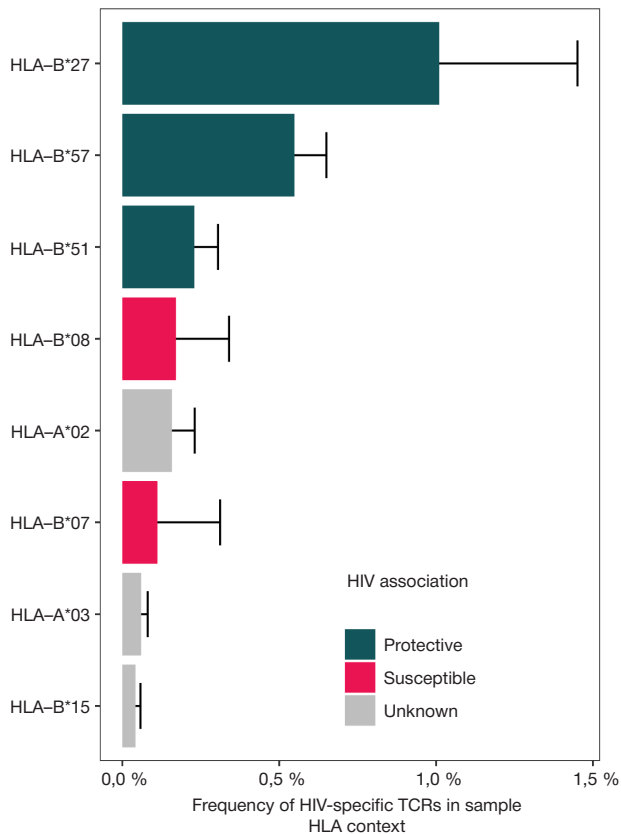


Fig. 3. The total frequency of TCR specific to HIV-epitopes represented by donor's HLA allele sets (data borrowed from Emerson et al.). Plotted along the X-axis is mean percent (for groups of individuals with the studied HLA allele) of the total number of reads for TCR sequences specific to HIV epitopes represented by a specific HLA allele. Standard deviation is provided for each value. The list of alleles was obtained by intersecting datasets from VDJdb and annotations from Emerson et al. study. Allele HLA-B*42 was excluded from the analysis, because it was represented by only one sample. Other alleles were present in more than 35 of 601 donors. Protective alleles and alleles associated with susceptibility to HIV were borrowed from Goulder and Walker's work [3]

the impact of CMV infection on the frequency of HIV-specific TCR in donors' repertoires. As shown in Fig. 2, the frequency of HIV-specific TCR was significantly higher for CMV-negative individuals regardless of the HIV epitope ($F = 495$, $p < 10^{-100}$, ANOVA). Of note, if TCR were not grouped based on the epitope they recognize, i.e. if the epitope-related difference in HIV-specific TCR frequencies was ignored, the result would be far less significant ($F = 61$, $p = 7 \times 10^{-15}$, ANOVA).

Information about the HLA haplotypes of the donors provided by Emerson et al. was used to estimate the number of HIV-specific TCR considering that the donor may have some of HLA alleles capable of representing an HIV epitope. As shown in Fig. 3, the largest proportion of HIV-specific TCR is observed for putatively protective B27, B57 and B51 HLA alleles [3]. For these 3 alleles the number of HIV-specific TCR is significantly higher than for 5 other alleles ($p < 0.01$, Tukey's post hoc test), except for the differences between alleles B51 and B08. It should be noted that we had to recruit a relatively small number of alleles for our study because there were no known HIV epitopes for other alleles in VDJdb.

Age-related changes in the structure of the T-cell repertoire were described in a number of previously published works [21] reporting the reduction of the observed repertoire diversity due to clonal expansions caused by chronic infections. Impoverished diversity results in the decreased proportion of T-cells (including the HIV-specific T-cells) capable of recognizing previously unencountered pathogens (Fig. 4):

$R = -0.35$ (Spearman's correlation coefficient here and else where; $p = 0.003$) for the data borrowed from Britanova et al. and $R = -0.20$ ($p = 0.001$) for CMV-negative individuals from Emerson's work. At the same time, CMV-positive patients demonstrate a less pronounced decrease in HIV-specific TCR ($R = -0.14$, $p = 0.03$). Massive clonal expansions ($> 5\%$ of TCR sequences) observed in CMV+ patients from the work by Emerson et al. can be explained by the cross-reactivity of HIV-specific clonotypes to CMV epitopes. Data borrowed from Britanova et al. illustrates reduced levels of HIV-specific T-cells in men ($R = -0.53$, $p = 0.002$) but not in women ($p = 0.22$), because the female cohort included centenarians (aged 80 to 100 years) whose repertoires are typically very specific [15].

DISCUSSION

Our findings indicate that the median frequency of HIV-specific T-cells in healthy individuals can vary by 1 or 2 orders of magnitude depending on the studied HIV epitope, which is consistent with estimates obtained by other researchers who used different techniques [11, 12, 22]. A far less pronounced variability is observed between the individuals for the frequencies of T-cells recognizing a particular epitope.

It should be noted that one of the major factors determining the frequency of a particular TCR sequence in the population is probability of its assembly during V(D)J recombination [7]. This process is well described by existing statistical models [23]. Recombination parameters do not vary a lot between across the population, which is consistent with our findings. Therefore, we infer that frequencies of HIV-specific T-cells calculated *in silico* are an important and reliable parameter of the magnitude of the immune response to particular epitopes at the population level.

To sum up, development of effective vaccination strategies should account for the pool of epitopes that may be represented in the individual HLA context and for the proportion of naive T-cells capable of recognizing these epitopes.

Frequencies of HIV-specific T-cells studied in the context of known HLAs vary significantly depending on the HLA. The highest frequencies of HIV-specific TCR were observed for protective alleles listed in [23].

Besides, we noticed that the proportion of HIV-specific T-cells goes down with age in individuals with CMV infection. These data are consistent with broader observations of the dynamics of T-cell repertoire diversity affected by age and chronic infections, including CMV [24–26]. Sex-based comparison of sequencing data borrowed from Britanova et al. and Emerson et al. works returns controversial results, because Britanova et al. study recruited female centenarians.

CONCLUSIONS

Our work demonstrates that sequencing of immune repertoires and the subsequent bioinformatic analysis allow in-depth study of antigen-specific T-cell populations. RepSeq is a valuable tool for estimating the frequency of HIV-specific T-cells in the repertoires of healthy donors that can be used to identify the factors affecting this frequency. As the VDJdb will grow to include more annotated sequences, our data will be supplemented with new HIV epitopes and HLA alleles. In the future, exploration of cell repertoires of HIV-infected donors carrying different HLA alleles, including elite controllers, will help us to identify those HIV-specific TCR present in the naive T-lymphocyte populations that have higher frequency and are associated with HIV inhibition.

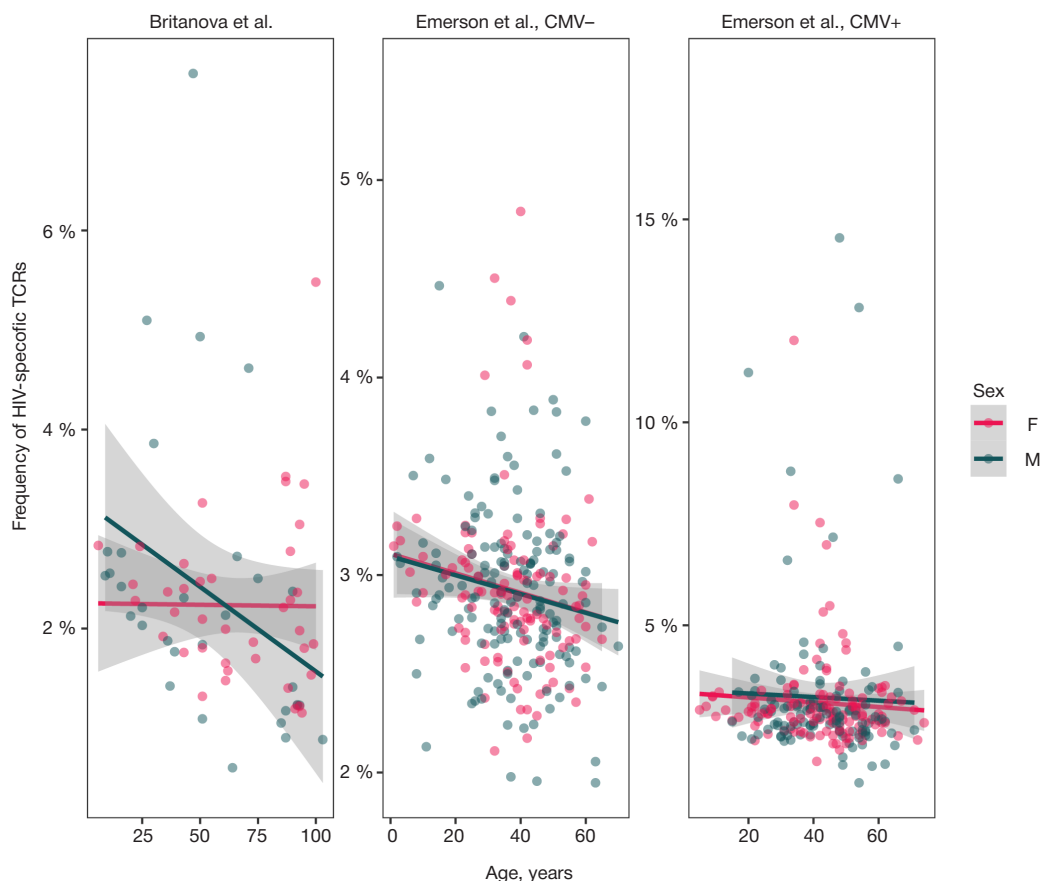


Fig. 4. Changes in the total frequency of HIV-specific TCR in donors of different sex and age. Data from Emerson et al. are distributed into groups based on the CMV status of the participants. The graph also shows results of linear modeling for HIV-specific TCR dependence on age for males and females

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