Diversity Complexity Index (DCI) for Spectratype/Immunoscope Analysis of the Expressed TCR Repertoire

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Abstract

Spectratyping, the most broadly utilized technique, measures TCR repertoire diversity based on variation in the lengths of RT-PCR products generated from the CDR3 region in each TCR Vβ family. This technique has been used successfully on samples from humans and mice, such as in vitro activated T cells, T cell lines, peripheral blood, central primary and peripheral lymphoid tissues, extra lymphoid sites of inflammation, biopsy samples or malignant tissue. General goal of quantitative analysis of spectratype data is to access the therapeutic benefits of treatment and its ability to maintain or even improve spectratype diversity. Most of analyzing spectratype profile methods is to measuring how objective spectratyping profile is diverted from the Gaussian or Gaussian like pattern of spectratyping in CDR3 region. Many times, we are more interested in answering the question if a certain intervention is improving/losing the repertoire diversity without assuming the Gaussian and Gaussian like pattern of spectratyping in CDR3 region.
However, due to the absence of standardized methods of analyzing and reporting the data, the interpretation of the data generated has remained an issue. We are proposing a diversity complexity index (DCI) that can evaluate not only degree of divergence between two spectratype profiles but also the direction of the spectratype profile change by considering both magnitude of peak and number of peaks.

Key Words: Spectratyping, Shannon-Jensen Divergence, Kullback-Leibler Divergence, TCR repertoire diversity, Immune reconstitution, Diversity Complexity Index

1. Background

Spectratyping, the most broadly utilized technique, measures TCR repertoire diversity based on variation in the lengths of RT-PCR products generated from the CDR3 region in each TCR Vβ family (Choi et al., 1989; Genevee et al., 1992; Pannetier et al., 1993; Gorski et al., 1994; Even et al., 1995).

This technique has been used successfully on samples from humans and mice, such as in vitro activated T cells, T cell lines, peripheral blood, central primary and peripheral lymphoid tissues, extra lymphoid sites of inflammation, biopsy samples or malignant tissue. Spectratyping is a well-established technique and many scoring methods have been described (reviewed in Miqueu et al.(2007)). The simplest, such as counting the numbers of peaks (taking the total or the average per Vβ family) have been useful in describing major differences in T cell receptor repertoire diversity. More complex methods involve converting the spectratype of each Vβ family into frequency distribution based on the proportion of each of the different CDR3 lengths (calculated as areas under the curve). Using this form of analysis, spectratypes have been compared in pairs, to identify clonal expansions. Several modifications in these protocols have been published to date including several methods of statistical analysis (Bercovici et al., 2000; Collette and Six, 2002; Hori et al., 2002; Killian et al., 2002; Miqueu et al., 2007). Due to the absence of standardized methods of analyzing and reporting the data, the interpretation of the data generated has remained an issue. Because of the random generation of a very large number of unique CDR3 regions, the spectratype of CDR3 fragment lengths will form a bell curve, a Gaussian like distribution. Clonal T cell expansions or severe cytoreduction results in skewing in the proportional distributions of CDR3 of different lengths (Sarfraz A. Memon 2012).

2. Review of quantitative analysis of spectratype data

The most important issue when analyzing spectratype data is determining how to assign the correct CDR3 amino acid base length observed in a given peak or band in a profile. Jeffrey R. Currier and Mary Ann Robinson (*Current Protocols in Immunology* (2000) 10.28.1-10.28.24) descried two ways to solve this. First, a TCR molecular clone from a given family can be sequenced to determine its CDR3 length in nucleotides and subsequently translated into amino acids. This clone can be diluted, amplified by PCR using the appropriate set of primers, and run on a spectratype gel in parallel with samples of the same V-gene family. The second method for determining the CDR3 length applies to running fluorescently labeled samples on an automated sequencing machine.

Gaussian and Gaussian like pattern of spectratyping in CDR3 region (8-12 amino acids correspond to 8-12 peaks) is considered as normal. Perturbed spectratype with less number of peaks and/or a single dominant peak (clonal expansion) consider as loosing repertoire diversity. This is bad for new infection with pathogens, and vaccination. Low diversity often means that we will be susceptible to infection.

General goal of quantitative analysis of spectratype data is to access the therapeutic benefits of treatment and its ability to maintain or even improve spectratype diversity. Most of analyzing spectratype profile methods is to measuring how objective spectratyping profile is diverted from the Gaussian or Gaussian like pattern of spectratyping in CDR3 region. Many times, we are more interested in answering the question if a certain intervention is improving/losing the repertoire diversity without assuming the Gaussian and Gaussian like pattern of spectratyping in CDR3 region.

The simplest, such as counting the numbers of peaks (taking the total or the average per Vβ family) have been useful in describing major differences in T cell receptor repertoire diversity. More complex methods involve converting the spectratype of each Vβ family into frequency distribution based on the proportion of each of the different CDR3 lengths. There, in summary, are two approaches analyzing spectratype profile. One is to adopt a divergence/similarity function for the probability distribution. The other is to develop a scoring.

Jeffrey R. Currier and Mary Ann Robinson(*Current Protocols in Immunology* (2000) 10.28.1-10.28.24) used Hamming distance (HD) to compare spectratype profile. The HD a metric, and it is a number used to denote the difference between two binary strings. The HD is also used in systematics as a measure of genetic distance. The HD for each profile is then calculated using the formula;

$$HD = 100 \times \sum_{i=1}^{n} |D|_i$$

Where D_i is the extent of perturbation or skewing at each CDR3 length (peak), $D_i = P_i$ (distribution 1) - P_i (distribution 2). Therefore, HD is the sum of the absolute differences of the distances between two probability distributions at each CDR3 length. Claude Sport è s et al. (2008) used a divergence score to explore if Administration of rhIL-7 in humans increases in vivo TCR repertoire diversity by preferential expansion of naive T cell subsets. The divergence score ” of each Vβ family from its counterpart Vβ normal donor standard as the sum of the absolute value of the divergence in proportional area at each peak. This divergence score is indeed identical to the HD.

Kelper et al (2005) introduced the Kullback-Leibler divergence (DKL) as quantification of spectratype data that can be used for the statistical hypothesis tests and parameter estimation. The Kullback-Leibler divergence (DKL) is generally a measure of difference between two probability functions, and in the appropriate context is a natural measure of deviation from maximum diversity. *KDL* is defined by

$$KDL(X,Y) = \sum O_i \ln(\frac{X_i}{Y_i})$$

where X_i is the observed relative peak area of distribution 1 and Y_i is the relative peak area of distribution 2. The K-L divergence is only defined if *X* and Y both sum to 1 and if $X_i = 0$ implies $Y_i=0$ for all *I*, that is, $0ln0 = 0, \ because \lim_{x\to o} xlogx = 0$

Some methods are more focus on the number of non-zero peak probability rather than the magnitude of peaks. The overall complexity within a Vβ TCR family and among the different V β TCR families was determined by counting the numbers of different peaks and determining their relative amplitude on the spectratype histogram (Catherine Bomberger, et al. 1998). The overall complexity within a Vβ TCR family and among the different Vβ TCR families was determined by counting the numbers of different peaks and determining their relative amplitude on the spectratype histogram. Their complexity score is defined by

$$Complexity\ Score\ (SC) = \frac{(\frac{MPHs}{TPHs})}{no.MP}$$

where MPHs is the sum of the heights of the major peaks and TPHs is the sum of all the peak heights (TPHs), and no.MP is the number of major peaks present. Major peaks were defined as those peaks on the spectratype histogram whose amplitude was at least 10% of the TPH.

Catherine Wu, et al. (2000) uses complex score. Sub-families were graded on a score of 0 to 8 based on the degree of complexity. Normal complexity is characterized by a Gaussian distribution of transcript sizes, which reflects the presence of polyclonal cDNA species and contains 8 to 10 peaks for each Vβ subfamily. A score of 0 was assigned if a subfamily was absent. A score of 1 was given if a subfamily demonstrated only a single monoclonal peak. A score of 2 was given for a biclonal profile. A subfamily was designated with a score of 3 if 3 peaks were present and so on. Finally, a score of 8

denoted a normal-appearing spectratype of 8 to 10 peaks with a complex, diverse, and polyclonal appearance. The overall spectratype complexity score per sample was calculated as the summation of the number of subfamilies (i.e., 26, because subfamilies 5 and 13 are represented by 2 sets of primers) per score category, with a maximum possible score of 208 (8×26). Peggs et al. (2003) developed a diversity scoring system was developed based on analysis of 11 cord blood (CB) and 12 normal adult BV spectratypes CB subfamily spectratypes demonstrated minor deviations from a Gaussian pattern consistent with current knowledge about germ line TCR rearrangements. Each BV subfamily was ascribed a score of 4 points for diversity (maximum score = #of subfamily× 4). One point was subtracted for each of the following: One or more peaks > 10% above the mean control value, Two or more peaks > 3 SD above the mean control value, less than six peaks, skewness and kurtosis values both > 3 SD outside the mean Control values.

3. Alternative Approaches

We described some scoring methods that can be used a quantified measure of spectratype diversity measures from the simplest such as counting the numbers of peaks to more complex methods that involve converting the spectratype of each Vβ family into frequency distribution. However, due to the absence of standardized methods of analyzing and reporting the data, the interpretation of the data generated has remained an issue. The KDL is generally a measure of difference between two probability functions, and in the appropriate context is a natural measure of deviation from maximum diversity. However, the KDL is not a metric. Since *KDL(X,Y)* is not generally same as *KDL(Y,X)*, KDL is not symmetric. Moreover, the KDL not always provides a finite value. This will cause some systematic problems for spectratyping data especially when some peaks are absent. Jianhua Lin (1991) introduced a measure based on Jensen's inequality and the Shannon entropy, an extension of the new measure, the Jensen-Shannon divergence, is derived. One of the major features of the Jensen-Shannon divergence **is** that we can assign different weights to the distributions involved according to their importance. This is particularly useful in the study of decision problems. The Shannon-Jensen divergence (JSD) can be used as a good alternative approach for the KDL because the JSD is symmetric and it is always finite value. And JSD provides quantitative measures of the discrepancies between two distribution functions as well. JSD is defined by

$$JSD(X,Y) = \lambda_1 KDL(X,Z) + \lambda_2 KDL(Y,Z)$$

where $Z = \frac{\lambda_1+\lambda_2}{2}(X+Y), KDL(.)$ is a Kullback Leibler Divergence, and $\lambda_1 + \lambda_2 = 1$.
The Jensen–Shannon divergence is bounded by log2. So the Jensen–Shannon divergence is bounded by 1, given that one uses the base 2 logarithm, that is, $0 \leq JSD(X,Y) \leq 1$. Since JSD is a metric, JSD satisfies non-negativity, identity of indiscernibles, and symmetric. In other words, the JSD satisfies the following conditions: *JSD(X,Y)≥0*, *JSD(X,Y) = 0* if and only *if X=Y*, and *JSD(X,Y)=JSD(Y,X)*.
Most of time, question of interest is to evaluate if an intervention improves or loses repertoire diversity spectratype diversity, and not necessary compare to normal Gaussian like spectratype profile. All measures we seen address whether spectratype profile was alternated but not a direction of this alternation. What we know is that number of peak plays very important role to define a direction of alternation because a perturbed spectratype with less number of peaks and/or a single dominant peak (clonal expansion) consider as loosing repertoire diversity.

We are proposing a diversity complexity index (DCI) that can evaluate not only degree of divergence between two spectratype profiles but also the direction of the spectratype profile change by considering both magnitude of peak and number of peaks. The diversity complexity index is defined by

$$DCI(X,Y) = \text{sign}(NP_X - NP_Y)\left\{\lambda_1\left[\frac{1}{2}\sum X_i \log_2\left(\frac{X_i}{Z_i}\right) + \frac{1}{2}\sum Y_i \log_2\left(\frac{Y_i}{Z_i}\right)\right] + \lambda_2\left[\frac{|NP_X - NP_Y|}{NP}\right]\right\}$$

where λ_1 and $\lambda_2$are weight whch are none negative constand with $\lambda_1 + \lambda_2 = 1$

NPX, NPY, and NP are number of positive picks in the profile X, number of positive picks in the profile Y, and number of CDR3 picks amino acid base length in the experiment, respectively. And Z is an average of X and Y, $Z = \frac{X+Y}{2}$. This can be simplified using JSD by

$$DCI(X,Y) = \text{sign}(NP_X - NP_Y)\left\{\lambda_1 JSD(X,Y) + \lambda_2\left[\frac{|NP_X - NP_Y|}{NP}\right]\right\}.$$

This Diversity Complexity Index is bounded by 1. Even though the DCI is not a metric, the DCI satisfies *-1 ≤DCI(X,Y)≤1, JSD(X,Y) = 0* if and only if *X=Y and* $NP_X = NP_Y$, and $|JSD(X,Y)| = |JSD(Y,X)|$.

4. Rhesus Monkey Spectratype Study

4.1. Experimental design and background

The Rhesus Macaque monkey study conducted at the Oregon National Primate Research Center (ONPRC) collaboration with University Center of Aging at University of Arizona to evaluate the therapeutic benefits of IL-7 administration and its ability to maintain or even improve spectratype diversity in old animals including questions of interests:

- Does IL-7 treatment improve spectratype profile in the adult?
- Does IL-7 treatment improve spectratype profile in the old?
- Any difference between the adult and the old?

We (Okoye et. al. Al., 2014) demonstrated that although thymic output is maintained at low levels in old RM, IL-7 therapy administered either as a repeated single dose or in a clustered dosing regimens induced only transient expansions of $CD4^+$ and $CD8^+$ T_N in old and adult RM. Strikingly however, this treatment had a more prolonged effect on T_{CM} expansion. We also confirm that the effects of IL-7 on T cell expansion are independent of a functioning thymus.

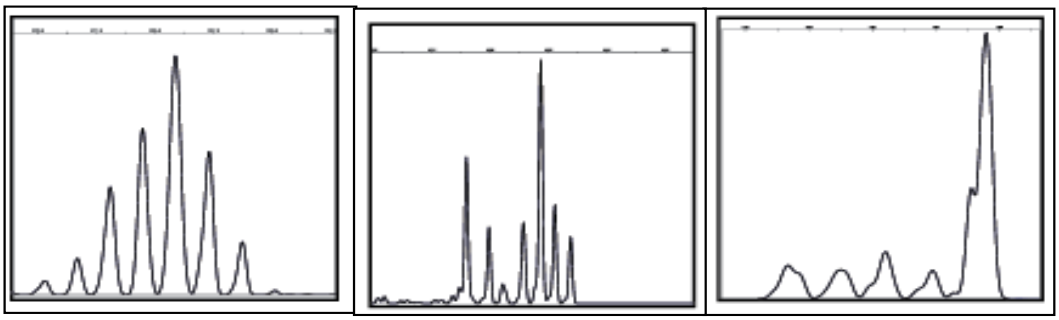

Figure 1. Gaussian and Gaussian like pattern of spectratyping in CDR3 region (8-12 amino acids correspond to 8-12 peaks) is considered as normal. Perturbed spectratype with less number of peaks and/or a single dominant peak (clonal expansion) consider as loosing repertoire diversity, and this is a bad for new infection with pathogens, and vaccination. As we age, T-cell diversity declines. Low diversity often means that we will be susceptible to infection.

4.2 Animals

In this study, we assessed the ability of therapeutically administered IL-7 to expand the T_N compartment by increasing T_N production in the thymus and/or expanding T_N pools in the periphery. To address this we used the NHP model of aged rhesus macaques (RM). This model matches favorably with humans as outbred genetics, exposure to environmental pathogens, and evolutionary relationship are all extremely close to humans (Nikolich-Zugich, 2007). Thus, it represents an excellent tool for studies with direct relevance to human immune senescence and immune reconstitution. There were two groups of animal selected based on age. Ages of adult monkeys are between 10 to 15years, and old animals are aged over 20 years old. Both groups were treated multiple times with IL-7 which is a cytokine important in maintaining T cell numbers and survival. The samples were isolated from the blood of old and adult animals before and after IL-7 therapy. IL-7 was administered subcutaneously with animals receiving 7/9 doses over a two year period. We analyzed data using DCI. There were two stage of data analysis. First stage is to evaluate the spectratype profile of each Vβ gene. The second stage is to explore effect of IL-7 administration on Vβ family.

4.3 Results

After quantification of individual spectratype profile for Signal processing of CDR3 length distribution as shown in figure 2.

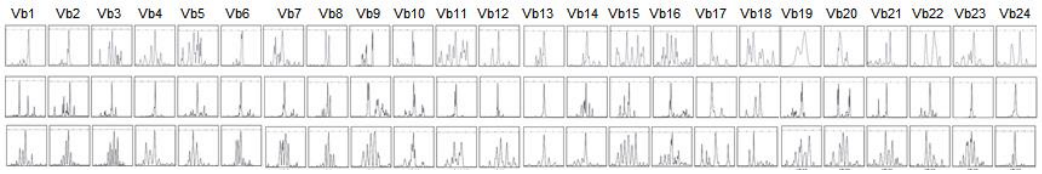

Figure 2. Examples of individual spectratype profile for Signal processing of CDR3 length distribution.

DCI values for individual level of each Vβ gene as well as an overall Vβ family were calculated to evaluate the efficacy of IL-7 treatment. We conventionally set $\lambda_1=\lambda_2=1/2$. Overall Vβ family diversity complexity index values by treatment scheme and T-cell subsets is summarized in the table 1. Positive DCI value indicates potential improvement on spectratype profile, in other words, administration of IL-7 increases TCR repertoire diversity, while negative DCI value indicates potential loss of spectratype profile diversity.

Table 1. Overall Vβ family diversity complexity index values by treatment scheme and T-cell subsets

GENE	Cohort 1 (Single Dose)		Cohort 2 (Cluster Dose)	
	Old	Adult	Old	Adult
CD4 Memory	1.374	2.521	4.664	3.884
CD4 Naive	2.854	2.535	-1.641	-4.291
CD8 Memory	4.342	4.538	4.623	4.237
CD8 Naive	-0.060	-0.689	0.543	2.361

IL-7 treatment looked more effective on TCR diversity for CD4 memory and CD8 memory with clustered dose. For example, average DCI score for old monkeys for single dose and cluster dose regimens are 1.374 and 4.664, respectively.

Table 2. Descriptive statistics of DCI (CD4 Memory)

Age	# of monkey	regime	# of Positive DCI	Mean	Median	Standard Deviation	95% Confidence Interval	
							Lower	Upper
Adult	4	Single	4	2.521	3.037	2.059	-0.756	5.798
	5	Cluster	5	3.884	4.274	1.826	1.616	6.151
Old	6	Single	5	1.374	2.274	2.181	-0.914	3.662
	6	Cluster	6	4.664	4.501	1.838	2.735	6.593

Even though there were too small sample to assess statistical significant of efficacy of treatment, but here is some operating characteristics. 5 out of 6 old monkey (83%) displayed positive DCI value.

Table 3. Descriptive statistics of DCI (CD8 Memory)

Age	# of monkey	regime	# of Positive DCI	Mean	Median	Standard Deviation	95% Confidence Interval	
							Lower	Upper
Adult	4	Single	4	4.538	4.313	1.372	2.355	6.722
	5	Cluster	5	4.237	4.518	2.303	1.378	7.097
Old	7	Single	7	4.342	4.101	2.620	1.918	6.765
	6	Cluster	6	4.623	4.580	1.639	2.903	6.344

Probability of more than equal to 5 out of 6 is 0.11 when the chance of being positive or being negative is 50%, that is, by chance. This is very conservative approach because TCR repertoire diversity declined with aging, assuming the chance of improving at 50% without any treatment would not be reasonable. For your information, if we arbitrarily assume that the chance of improving TCR repertoire diversity is 30%, then the

probability more than equal to 5 out of 6 is 0.04. Table 2 also suggests that cluster dose scheme looked more effective for both adult and old rhesus macaque.
Table 3 suggests that IL-7 treatment helps to improve CD8 memory cell TCR repertoire diversity on both adult and old rhesus macaques regardless of dosage schemes. Table 4 displays average DCI value of individual spectratype profile for each Vβ gene.

Table 4. Average DCI value of CD8 Memory individual spectratype profile for each Vβ gene.

GENE	Cohort 1 (Single Dose)		Cohort 2 (Cluster Dose)	
	Old Average	Adult Average	Old Average	Adult Average
Vb1	0.041	0.058	0.517	0.296
Vb10	0.392	0.399	0.152	0.133
Vb11	-0.150	-0.007	-0.175	0.213
Vb12	-0.317	0.229	0.465	0.121
Vb13	-0.597	-0.567	-0.229	-0.331
Vb14	0.224	0.322	0.180	0.500
Vb15	0.326	0.302	0.017	0.324
Vb16	0.242	0.305	0.184	0.277
Vb17	0.300	0.092	0.244	0.137
Vb18	0.107	-0.035	0.121	-0.185
Vb19	0.250	0.017	0.344	-0.407
Vb2	0.414	0.307	0.034	0.112
Vb20	0.422	0.397	0.333	0.271
Vb21	0.288	0.032	0.035	0.300
Vb22	0.157	0.108	0.250	0.507
Vb23	0.162	0.195	0.112	0.317
Vb24	0.233	0.506	0.247	-0.087
Vb3	0.329	0.226	0.036	0.250
Vb4	0.132	0.457	0.455	0.114
Vb5	0.335	0.473	0.231	0.457
Vb6	0.328	0.315	0.237	0.487
Vb7	0.378	0.212	0.317	0.176
Vb8	0.155	0.174	0.276	0.240
Vb9	0.276	0.437	0.355	0.279
Whole Family	4.342	4.538	4.623	4.237

Most of DCI values of individual spectratype profile for each Vβ gene were positive. Even though there was lack of sample size to assess statistical significant of the efficacy of treatment, the analysis using DCI is consistent to profiles of spectratype and successfully evaluate the therapeutic benefits of IL-7 administration and its ability to maintain or even improve spectratype diversity in old animals as well as adult animals as seen in the TCR profiles.

Acknowledgements

This publication is made possible with NIH/NIAID N01-AI-00017 (Protective Immunity in Special population), 2P30 CA069533-14 (Knight Cancer Institute from the National Cancer institute, and 7005-11 (Specialized Center of Research *by* The Leukemia and Lymphoma Society.

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