Laboratory Investigation
J Korean Neurosurg Soc 64 (4) : 505-513, 2021
https://doi.org/10.3340/jkns.2020.0214
pISSN 2005-3711 eISSN 1598-7876

Profiling of T Cell Receptor β-Chain Complimentary Determining Regions 3 Repertoire in Subarachnoid Hemorrhage Patients Using High-Throughput Sequencing

Bong Jun Kim,[1,*] Jun Hyong Ahn,[2,*] Dong Hyuk Youn,[1] Jin Pyeong Jeon[1,2,3]

Institute of New Frontier Stroke Research,[1] Hallym University College of Medicine, Chuncheon, Korea
Department of Neurosurgery,[2] Hallym University College of Medicine, Chuncheon, Korea
Genetic and Research Inc.,[3] Chuncheon, Korea

Objective : The adaptive immune response following subarachnoid hemorrhage (SAH) is not well understood. We evaluated and compared the T cell receptor (TCR) immune repertoire of good-grade and poor-grade SAH patients to elucidate the T cell immunology after ictus.

Methods : Peripheral blood from six SAH patients was collected at two different times, admission and at the 7-day follow-up. Composition and variation of the TCR β-chain (TCRB) complimentary determining regions (CDR) 3 repertoire was examined using high-throughput sequencing; the analysis was based on sampling time and disease severity (good vs. poor-grade SAH).

Results : Clonality at admission and follow-up were 0.059 (0.037–0.038) and 0.027 (0.014–0.082) (median, 25th–75th percentile). Poor-grade SAH (0.025 [0.011–0.038]) was associated with significantly lower clonality than good-grade SAH (0.095 [0.079–0.101]). Poor-grade SAH patients had higher diversity scores than good-grade SAH patients. CDR length was shorter in good-grade SAH vs. poor-grade SAH. Differences in clonotype distribution were more prominent in TCRBV gene segments than TCRBJ segments. TCRBV19-01/TCRBJ02-04 and TCRBV28-01/TCRBJ02-04 were the most increased and the most decreased V-J pairs in the 7-day follow-up compared to admission in good-grade SAH. The most increased and decreased V-J pairs in poor-grade SAH patients were TCRBV28-01/TCRBJ02-06 and TCRBV30-01/TCRBJ02-04, respectively.

Conclusion : The TCRB repertoire is dynamic in nature following SAH. TCRB repertoire may facilitate our understanding of adaptive immune response according to SAH severity.

Key Words : Subarachnoid hemorrhage · T cell receptor beta · Immunity · High-throughput sequencing.

INTRODUCTION

The adaptive immune response is mediated by diverse receptors on the surface of T cells that allow for recognition of pathogens[8]. Accordingly, identification of the T cell receptor (TCR) repertoire can be used to monitor the host immune re-

• Received : July 27, 2020 • Revised : September 18, 2020 • Accepted : October 7, 2020
• Address for reprints : **Jin Pyeong Jeon**
Department of Neurosurgery, Hallym University College of Medicine, 77 Sakju-ro, Chuncheon 24253, Korea
Tel : +82-33-240-5171, Fax : +82-33-240-9970, E-mail : jjs6553@daum.net, ORCID : https://orcid.org/0000-0001-8543-6855

*Bong Jun Kim and Jun Hyong Ahn contributed equally to this work and should be considered co-first authors.

sponse. Characterization of TCR repertoires have proven useful in cancer and autoimmune disease patients who received immunotherapy[34]. Sims et al.[30] sequenced TCRs of glioma α- and β-chains. They showed that more divergent VJ-independent repertoires are associated with tumors than non-neoplastic tissues. Yu et al.[34] evaluated the associations between TCR repertoire and symptom remission in systemic lupus erythematosus (SLE) patients treated with glucocorticoids. They suggested that monitoring of the TCR complimentary determining region (CDR) 3 is related to prognosis in SLE, suggesting that it mirrors therapeutic efficacy. T cells are also actively involved in atherosclerosis[14,22]. Jia et al.[14] reported epigenetic suppression of forkhead box that was related with a decrease in regulatory T cells, resulting in an increased risk of atherosclerosis. In addition, the T cell response is involved in the initiation of plaque disruption[10].

SAH occurs spontaneously via ruptured aneurysm, arteriovenous malformation (AVM), or traumatic brain injury. The mortality rate of SAH due to ruptured cerebral aneurysm exceeded 20%[21,33]. Although an aneurysm itself may be successfully occluded, neurologic complications can occur due to direct brain injury and vascular dysregulation[26]; such complications are associated with poor neurologic outcomes if not promptly treated. Consequently, SAH patients require multimodal monitoring in the neurointensive care unit for early detection of neurologic complications and subsequent optimal intervention[26]. In attempts to understand the pathophysiology of SAH, many researchers have focused on genomic and proteomic analyses; by comparison, the adaptive immune response has not been well studied[1]. Recently, we reported the TCR repertoire for the first time in SAH patients with and without delayed cerebral ischemia (DCI)[18]. In addition, we further evaluated the T cell immunology based on SAH severity to delineate SAH pathogenesis.

MATERIALS AND METHODS

Study population

This investigation was approved by the Institutional Review Board of the participating hospital (No. 2016-3, 2017-9, and 2018-6). Informed consent was received from all patients or their relatives. The study cohort was derived from the participating hospital's stroke database, which is part of an ongoing prospective, observational project in the regional medical center of the district; all patients presented to the hospital between March 2016 and December 2019[7,11,16,19,20,27,33]. We selected SAH patients based on the following conditions : 1) age over 18 years old; 2) spontaneous SAH due to ruptured aneurysms with saccular appearance; and 3) treatment with coil embolization. The exclusion criteria were : 1) fusiform, dissection, and infectious aneurysms; 2) angiogram-negative SAH; and 3) SAH concomitant with other cerebrovascular diseases including AVM and dural arteriovenous fistula[17].

We aimed to compare the composition and variation of the TCR β-chain (TCRB) CDR3 repertoire between the day of admission and at the time of follow-up, 7 days afterwards. Additionally, we divided patients into two groups based on SAH severity, good- and poor-grade, and further compared TCRB repertoire between these groups. Good-grade SAH was defined as initial Hunt and Hess (H-H) grade less than III. Clinical and radiologic medical information were reviewed. Clinical outcome was assessed using modified Rankin Scale (mRS) score 6 months after SAH.

DNA extraction and high-throughput sequencing

Peripheral blood samples were prepared on the day of admission and follow-up. Genomic DNA was extracted from whole blood using the QIAamp DNA Blood Midi Kit (Qiagen, Hilden, Germany), quantified with a DropSense96, and diluted for library preparation in buffer according to a standard. Then, sample data was generated using the immunoSEQ assay (Adaptive Biotechnologies, Seattle, WA, USA). The somatically rearranged locus CDR3 region was amplified using a bias-controlled multiplex polymerase chain reaction (PCR)[4,18,29]. Specifically, the first PCR was performed for every V and J gene segments using forward and reverse amplification primers, and the hypervariable CDR3 was amplified. Then, proprietary barcode sequence and Illumina adapter sequences (https://support.illumina.com) were performed in the second PCR[28]. CDR3 libraries were sequenced on an Illumina instrument according to the manufacturer's instructions.

Analysis of TCRB repertoire

The raw Illumina sequence reads were demultiplexed and then further processed in the following steps : removal of the adapter and primer sequences, identification and correction of technical errors, and removal of primer dimer, germline,

 https://doi.org/10.3340/jkns.2020.0214

and other contaminant sequences. Then, the data were filtered and clustered to merge closely-related sequences using relative frequency ratio and modified nearest-neighbor algorithm. In the variable region of the chains, antibody is generated by the combination of the gene fragments, V, D, and J genes. The V, D, and J regions are separated and encoded by single or multiple axons[3]. Accordingly, the V, D, and J genes were defined based on annotation in compliance with the IMGT database (www.imgt.org). The biological CDR3 locus sequences were normalized and quantified[4,18]. The immunoSEQ Analyzer toolset was used for data analysis. The results of comparisons are described as median (25th–75th percentile) using the Mann-Whitney U test. *p* values less than 0.05 are considered statistically significant. Analyses were conducted with SPSS ver. 19 (SPSS, Chicago, IL, USA)[13].

RESULTS

Baseline characteristics of the study

TCRB CDR3 repertoires from six SAH patients were analyzed and compared at two different time points, admission and 7-day follow-up. Three patients presented with good-grade SAH, while the three others had poor-grade SAH. The median age was 53.5 years (range, 41–77). Four of the patients were female (66.7%). Most aneurysms (n=5, 83.3%) were lo-

Table 1. Clinical characteristics of the enrolled patients

Pt No.	Age (years)	Sex	Hunt and Hess grade*	Fisher grade	Aneurysm site	DCI	6-month mRS
1	61	F	II	2	MCA	No	0
2	50	F	II	3	MCA	Yes	0
3	77	F	III	3	A-com	No	0
4	47	M	IV	4	PICA	No	3
5	57	M	IV	4	A-com	No	0
6	41	F	IV	3	MCA	Yes	1

*Poor-grade SAH patients are defined as those presenting with Hunt and Hess grade IV. Pt : patient, DCI : delayed cerebral ischemia, mRS : modified Rankin Scale, F : female, MCA : middle cerebral artery, A-com : anterior communicating artery, M : male, PICA : posterior inferior cerebellar artery

Table 2. TCRB CDR3 repertoire – total sequences, unique sequences, and clonality – in patients with subarachnoid hemorrhage at two times, admission and follow-up

Pt No.	Nucleic acid*			Total	Frame	Sequences (amino acid)†	
	Group	Total	Unique		Unique	Clonality	Max frequency (%)
1	Adm	6762	5721	4961	4069	0.094	5.74
	F/U	8187	6858	5919	4803	0.096	6.44
2	Adm	23275	19296	13403	10858	0.150	4.74
	F/U	3431	2826	3023	2476	0.013	0.61
3	Adm	5213	4222	4698	3788	0.007	0.35
	F/U	4171	3334	3565	2837	0.015	0.48
4	Adm	1797	1509	1318	1082	0.073	5.56
	F/U	4019	3360	2816	2291	0.102	9.16
5	Adm	3743	2901	2808	2229	0.044	2.99
	F/U	8032	6244	5981	4743	0.039	1.97
6	Adm	2261	1721	1785	1380	0.035	3.05
	F/U	4648	3657	4174	3271	0.009	0.47

*The number of nucleic acid sequences. †The number of amino acid sequences. TCRB : T cell receptor β-chain, CDR : complimentary determining regions, Pt : patient, Adm : admission, F/U : follow-up

cated in the anterior circulation. Two patients experienced severe DCI and recovered well after chemical angioplasty. Three patients with good-grade SAH recovered well without significant disability. However, one out of three patients (33.3%) exhibited poor neurologic outcome defined by mRS score of 3 (Table 1).

Sequence, clonality, and CDR3 lengths of TCRB

Variables such as total and unique sequences, clonality, and frequency of the TCRB CDR3 repertoire were measured (Table 2). Clonality using the Shannon index at admission and follow-up were 0.059 (0.037–0.038) and 0.027 (0.014–0.082) (p=0.699), respectively. Clonality of poor-grade (0.025 [0.011–

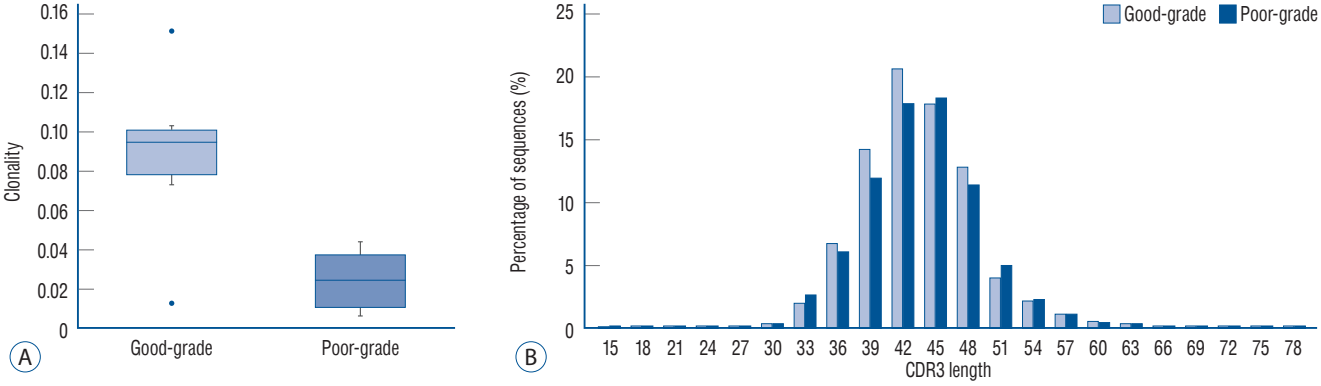

Fig. 1. Comparison of TCRB clonality (A) and CDR3 length repertoire (B) according to SAH severity, good- vs. poor-grade. Good-grade SAH patients had higher clonality and shorter CDR3 length than did poor-grade SAH patients. CDR : complimentary determining regions, TCRB : T cell receptor β-chain, SAH : subarachnoid hemorrhage.

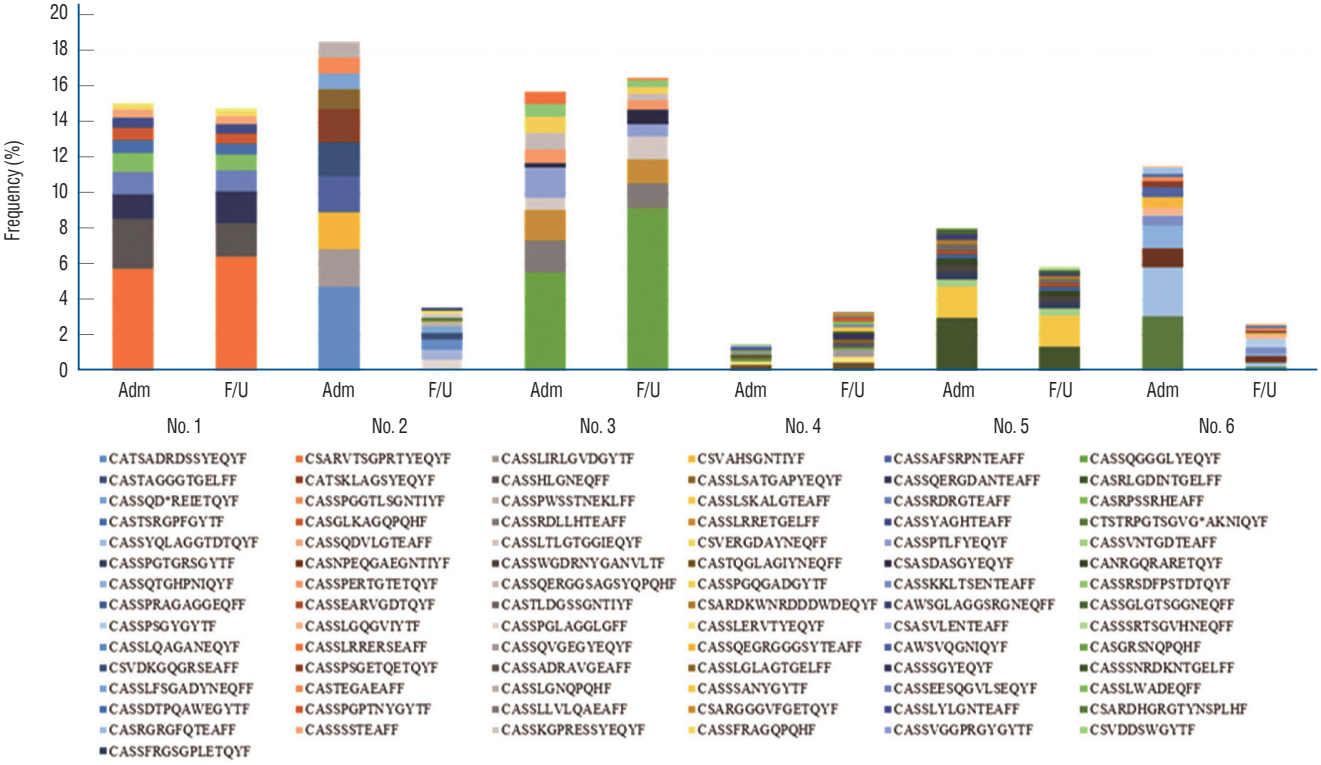

Fig. 2. Quantification of TCRB CDR3 sequences in each patient between the day of admission and the 7-day follow-up. The 10 most common sequences are presented in different colors. Patients who presented with poor-grade SAH tended to show a low proportion of the top 10 most common sequences compared to those with good-grade SAH at admission. Adm : admission, F/U : follow-up, TCRB : T cell receptor β-chain, CDR : complimentary determining regions, SAH : subarachnoid hemorrhage.

 https://doi.org/10.3340/jkns.2020.0214

0.038]) SAH was significantly lower than that of good-grade SAH (0.095 [0.079–0.101]; p=0.026; Fig. 1A).

Read frequency of ≤0.005% to <0.05% dominated the clonal expansion, but the changes between admission and follow-up did not show a definite association (Supplementary Table 1). We ranked TCRB clonotype frequencies and compared repertoire diversity using the Simpson diversity index (D)[5], where a score close to one indicates high diversity (Supplementary Fig. 1). Overall, the diversity indices ranged from 0.993 to 0.999 at admission and from 0.987 to 0.999 at follow-up. Poor-grade SAH patients had diversity scores of 0.998 and 0.999, which were higher than the scores of good-grade SAH patients.

CDR3 lengths ranged from 15 to 78 nucleotides, with a peak at 42 in good-grade and at 45 in poor-grade SAH patients. The five most common CDR3 lengths were 36, 39, 42, 45, and 48 nucleotides. The results are calculated as the average of five individuals. Good-grade SAH cases tended to have shorter CDR lengths compared to poor-grade SAH (Fig. 1B). The ten most expanded sequences were presented and compared in terms of amino acid levels (Fig. 2)[32]. The distribution of the top ten most common sequences was different in each patient. Overall, poor-grade SAH patients had a lower proportion of the top ten most common sequences than did good-grade SAH patients.

Distribution of TCRBV and TCRBJ

We analyzed the distribution of the unique clonotypes in both the TCRBV and TCRBJ gene segments. Overall, TCRBV gene segments from the follow-up day had different clonotypes than did those from the admission day, but the distribution of TCRBJ gene segments was similar between days. Compared to good-grade SAH, poor-grade SAH tended to exhibit a great number of different clonotypes of the TCRBV gene segments (Fig. 3). We analyzed the relative frequencies of V-J combinations using different colors representing disease severity. We obtained 754 annotated V-J pairs. TCRBV21-01/TCRBJ01-05, TCRBV21-01/TCRBJ01-04, and TCRBV21-01/TCRBJ02-04 were the most commonly increased V-J pairs in poor-grade SAH compared to good-grade SAH patients (Supplementary Fig. 2). We further compared the V-J combinations at two different time points (admission and 7-day follow-up) in good-grade and poor-grade SAH patients, respectively. The results showed that TCRBV19-01/TCRBJ02-04 was the most

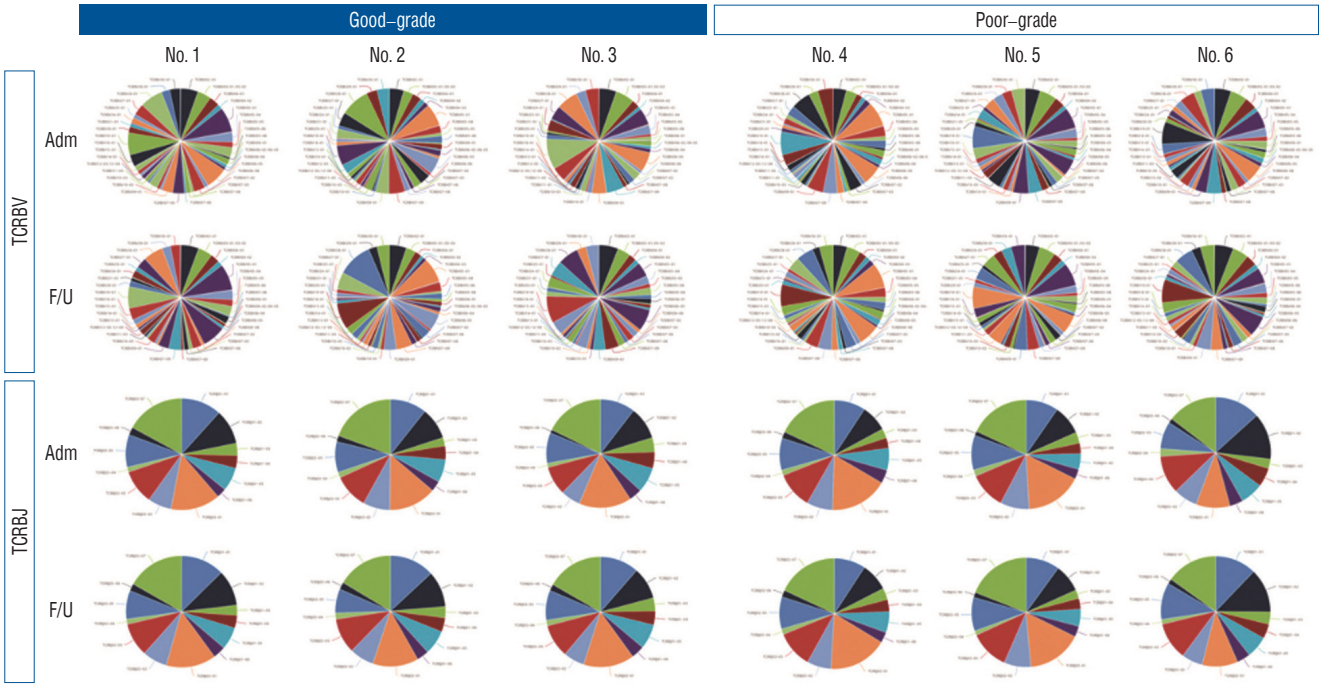

Fig. 3. Distribution and change in the unique clonotypes of the TCRBV and TCRBJ genes in patients with SAH. Compared to TCRBJ, TCRBV showed different distributions between the day of admission and the 7-day follow-up. Different colors are used to represent gene frequencies in each patient. TCRBJ : T cell receptor β chain J segment, TCRBV : T cell receptor β chain V segment, Adm : admission, F/U : follow-up, SAH : subarachnoid hemorrhage.

increased V-J pair and TCRBV28-01/TCRBJ02-04 was the most decreased V-J pair in good-grade SAH. Regarding poor-grade SAH patients, the most increased and decreased V-J pairs were TCRBV28-01/TCRBJ02-06 and TCRBV30-01/TCRBJ02-04, respectively (Fig. 4).

DISCUSSION

T cells recognize antigens with the major histocompatibility complex-TCR complex. The TCR repertoire mainly relies on diversity in the α- and β-chains[8]. Among the hypervariable regions of CDRs chains, CDR3 has been targeted for high-throughput immune sequencing due to its diversity[8,32]. Chang et al.[5] compared TCRB repertoires in rheumatoid arthritis patients who received different medications. In their study, the diversity of the TCRB repertoire varied according to different biological medications and was inversely correlated with disease activity[5]. Sun et al.[31] reported that TCRB diversity was lower overall in neonatal sepsis than in controls, with different clonotypes and V-J gene combinations in the two. In addition, shortening of CDR3 was suggested to increase autoimmune disease risk by increased self-recognition during thymic T cell development in type 1 diabetus mellitus[9]. Therefore, the TCR repertoire can function as a mirror of the health of the host, a marker of disease severity, or a marker of biological response for immunotherapy[2].

It has been postulated that SAH can spark innate and adap-

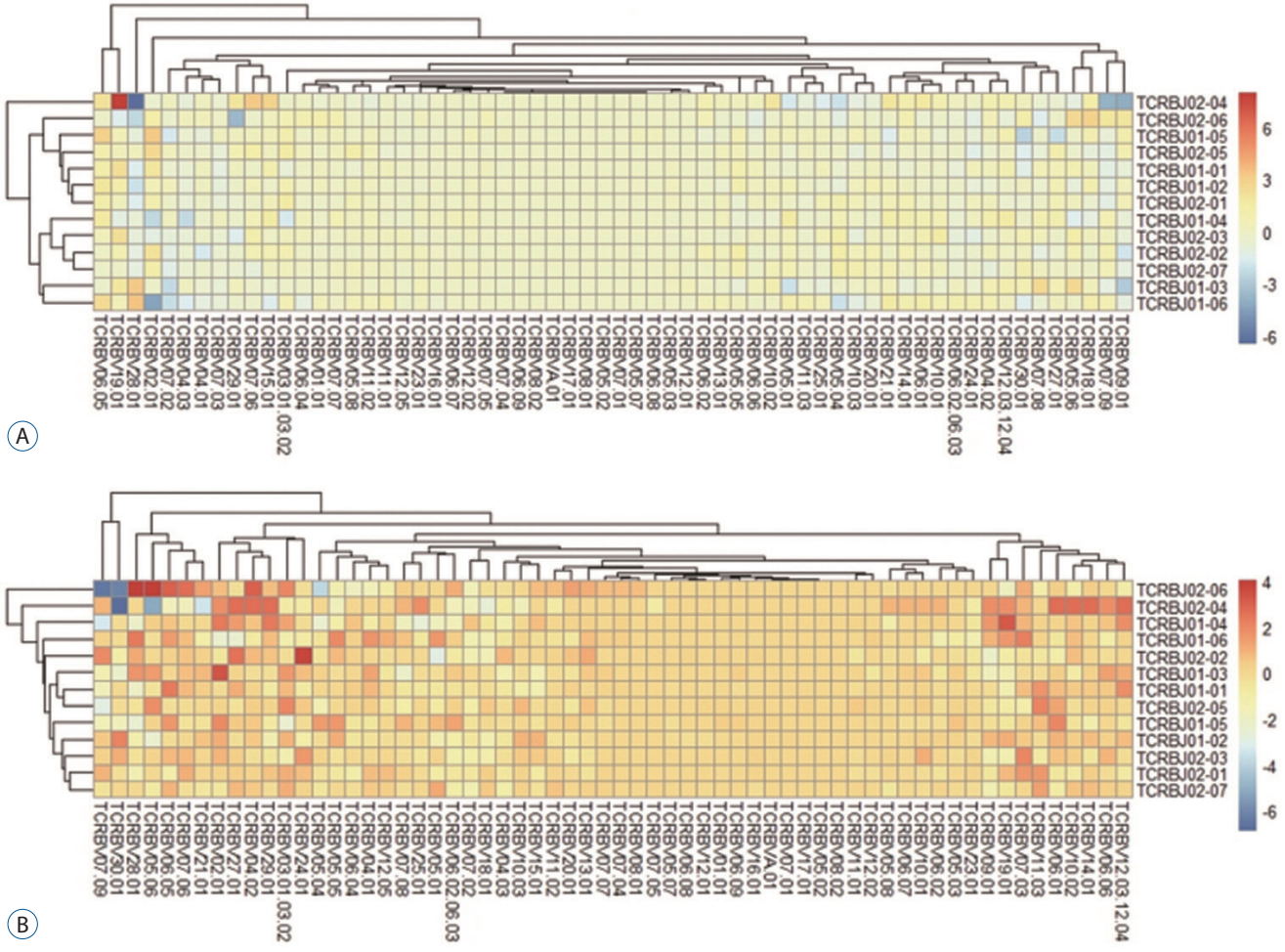

Fig. 4. Comparison of average TCRB V-J gene utilization at admission and during the 7-day follow-up according to SAH severity of good (A) and poor (B)-grade. V gene and J gene segments are arranged on the x- and y-axis. Different colors indicate the differences in gene frequencies of poor-grade SAH patients compared with good-grade SAH patients. TCRB : T cell receptor β-chain, SAH : subarachnoid hemorrhage.

 https://doi.org/10.3340/jkns.2020.0214

tive immune responses[25]. The study of immune response in SAH has generally focused on pro-inflammatory cytokines, which are responsible for brain edema after hemorrhagic insult, and treatments to alleviate the condition[6]. Hemorrhage leak into the subarachnoid space can trigger recruitment of leukocytes and inflammatory responses[15]. SAH patients exhibited increased levels of interleukin (IL)-6 and soluble IL-2 receptor in the cerebrospinal fluid[24]. Chen et al.[6] reported a beneficial effect of melatonin on preservation of tight junction proteins in the blood-brain barrier, resulting in decreased brain edema in an experimental SAH model in rats. In their study, the levels of pro-inflammatory cytokines such as IL-6, IL-1β, and tumor necrosis factor-α were suppressed after melatonin injection. Moraes et al.[25] also reported higher levels of innate immune cells such as $CD14^{++}$, $CD16^{+}$, and $CD69^{+}$ following SAH. However, the adaptive immune response, and TCRB repertoire in particular, has not been well studied in SAH patients. In this study, we explored the composition and variation of the TCRB CDR3 repertoire to understand T cell-mediated adaptive immunity after SAH. TCRB CDR3 repertoires are dynamic in nature according to disease severity. Poor-grade SAH patients showed significantly decreased clonality and increased diversity as compared with good-grade SAH patients, as well as longer CDR3 amino acid lengths. Interestingly, disease severity was associated with differences in VJ gene usage of TCRB and CDR3 lengths, which can affect TCR rearrangement. Therefore, further studies on the role of individual clonotypes in the development of clinical complications such as DCI or SAH-induced immunocompromised states are necessary.

In this study, we only included SAH patients who received endovascular coil embolization though the transfemoral approach, not those with craniotomy and clipping. The host immune response to tissue injury can affect systemic inflammatory and anti-inflammatory responses[23]. Marik and Flemmer[23] reported increased T-helper 2 cells which can result in impaired cell-mediated immunity after physical injury. Compared to coil embolization, clipping is associated with concerns about infection of the surgical site and tissue injury. Accordingly, we only sequenced and analyzed the TCRB repertoire in SAH patients with coiling to reduce inherent bias from different treatment methods. Therefore, patients who underwent craniotomy and clipping may have different TCRB CDR3 repertoires following SAH, which requires additional investigation.

The study limitations are as follows. First, the small sample size may be a concern, although this is the largest series to date involving SAH patients. Most previous studies enrolled less than 10 patients due to economic concerns[5,12]. Our previous study analyzing the TCRB repertoire in DCI also included five SAH patients[18]. Nevertheless, economic concerns cannot justify the results of the study. Accordingly, further studies are needed to investigate the adaptive immune response in a large number of the SAH patients to validate our findings. Second, we did not evaluate the TCR α-chain. Although most research has been done on either the α- or β-chain, one at a time[8], quantification of both the α- and β-chains can more accurately determine the TCR repertoire. Third, we have focused on the clinical grading system of H-H grade, but not Fisher grade. T cell adaptive immune response via extravasation of blood can trigger additional inflammatory response. We have analyzed and compared the average TCRB V-J gene utilization according to Fisher grades, II and III (A) and IV (B) (Supplementary Fig. 3). TCRBV19-01/TCRBJ02-04 was the most increased V-J pair in Fisher grades II and III in the 7-day follow-up and TCRBV28-01/TCRBJ02-04 was the most decreased V-J pair. In case of Fisher grade IV, TCRBV28-01/TCRBJ02-06 and TCRBV30-01/TCRBJ02-04 were the most increased and decreased V-J pairs, respectively. Nevertheless, the small number of enrolled patients may be a concern for the interpretation, suggesting the need for further TCRB studies focusing on Fisher grade. Fourth, different drug regimens, which possibly affect immunity, cannot accurately reflect the adaptive immune response. Accordingly, additional multicenter studies based on drug regimen are needed to evaluate TCRB repertoire.

CONCLUSION

The TCRB repertoire has a dynamic nature following SAH. Poor-grade SAH patients displayed a reduction in clonality and an increase in diversity and CDR3 length. Monitoring TCRB CDR3 repertoires can provide a better understanding of the adaptive immune response and could be useful for identification of therapeutic targets in SAH patients.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

INFORMED CONSENT

Informed consent was obtained from all individual participants included in this study.

AUTHOR CONTRIBUTIONS

Conceptualization : JPJ
Data curation : JHA, BJK, DHY
Formal analysis : BJK
Funding acquisition : JPJ
Methodology : BJK, JPJ
Project administration : JPJ
Visualization : BJK
Writing - original draft : JPJ, BJK
Writing - review & editing : JPJ

ORCID

Bong Jun Kim https://orcid.org/0000-0003-3374-5554
Jun Hyong Ahn https://orcid.org/0000-0002-8529-6757
Dong Hyuk Youn https://orcid.org/0000-0003-2259-1844
Jin Pyeong Jeon https://orcid.org/0000-0001-8543-6855

• Acknowledgements

This research was supported by the National Research Foundation of Korea funded by the Ministry of Education (2020R1l1A3070726), Hallym Research Fund, and Hallym University Research Fund (HURF-2019-29).

• Supplementary materials

The online-only data supplement is available with this article at https://doi.org/10.3340/jkns.2020.0214.

References

1. Akamatsu Y, Pagan VA, Hanafy KA : The role of TLR4 and HO-1 in neuroinflammation after subarachnoid hemorrhage. **J Neurosci Res 98 :** 549-556, 2020
2. Attaf M, Huseby E, Sewell AK : αβ T cell receptors as predictors of health and disease. **Cell Mol Immunol 12 :** 391-399, 2015
3. Boyd SD, Joshi SA : High-throughput DNA sequencing analysis of antibody repertoires. **Microbiol Spectr 2**, 2014
4. Carlson CS, Emerson RO, Sherwood AM, Desmarais C, Chung MW, Parsons JM, et al. : Using synthetic templates to design an unbiased multiplex PCR assay. **Nat Commun 4 :** 2680, 2013.
5. Chang CM, Hsu YW, Wong HS, Wei JC, Liu X, Liao HT, et al. : Characterization of T-cell receptor repertoire in patients with rheumatoid arthritis receiving biologic therapies. **Dis Markers 2019 :** 2364943, 2019
6. Chen J, Chen G, Li J, Qian C, Mo H, Gu C, et al. : Melatonin attenuates inflammatory response-induced brain edema in early brain injury following a subarachnoid hemorrhage: a possible role for the regulation of pro-inflammatory cytokines. **J Pineal Res 57 :** 340-347, 2014
7. Cho YD, Kim SE, Lim JW, Choi HJ, Cho YJ, Jeon JP : Protected versus unprotected carotid artery stenting : meta-analysis of the current literature. **J Korean Neurosurg Soc 61 :** 458-466, 2018
8. Dupic T, Marcou Q, Walczak AM, Mora T : Genesis of the αβ T-cell receptor. **PLoS Comput Biol 15 :** e1006874, 2019
9. Gomez-Tourino I, Kamra Y, Baptista R, Lorenc A, Peakman M : T cell receptor β-chains display abnormal shortening and repertoire sharing in type 1 diabetes. **Nat Commun 8 :** 1792, 2017
10. Hansson GK : Inflammation, atherosclerosis, and coronary artery disease. **N Engl J Med 352 :** 1685-1695, 2005
11. Hong EP, Kim BJ, Cho SS, Yang JS, Choi HJ, Kang SH, et al. : Genomic variations in susceptibility to intracranial aneurysm in the Korean population. **J Clin Med 8 :** 275, 2019.
12. Hou X, Wang M, Lu C, Xie Q, Cui G, Chen J, et al. : Analysis of the repertoire features of TCR beta chain CDR3 in human by high-throughput sequencing. **Cell Physiol Biochem 39 :** 651-667, 2016
13. Jeon JS, Ahn JH, Moon YJ, Cho WS, Son YJ, Kim SK, et al. : Expression of cellular retinoic acid-binding protein-I (CRABP-I) in the cerebrospinal fluid of adult onset moyamoya disease and its association with clinical presentation and postoperative haemodynamic change. **J Neurol Neurosurg Psychiatry 85 :** 726-731, 2014
14. Jia L, Zhu L, Wang JZ, Wang XJ, Chen JZ, Song L, et al. : Methylation of FOXP3 in regulatory T cells is related to the severity of coronary artery disease. **Atherosclerosis 228 :** 346-352, 2013
15. Kamel H, Iadecola C : Brain-immune interactions and ischemic stroke: clinical implications. **Arch Neurol 69 :** 576-581, 2012
16. Kim BJ, Kim Y, Hong EP, Jeon JP, Yang JS, Choi HJ, et al. : Correlation between altered DNA methylation of intergenic regions of ITPR3 and development of delayed cerebral ischemia in patients with subarachnoid hemorrhage. **World Neurosurg 130 :** e449-e456, 2019
17. Kim BJ, Kim Y, Youn DH, Park JJ, Rhim JK, Kim HC, et al. : Genome-wide blood DNA methylation analysis in patients with delayed cerebral isch-

emia after subarachnoid hemorrhage. **Sci Rep 10 :** 11419, 2020
18. Kim BJ, Youn DH, Kim Y, Jeon JP : Characterization of the TCR β Chain CDR3 repertoire in subarachnoid hemorrhage patients with delayed cerebral ischemia. **Int J Mol Sci 21 :** 3149, 2020
19. Kim CH, Jeon JP, Kim SE, Choi HJ, Cho YJ : Endovascular treatment with intravenous thrombolysis versus endovascular treatment alone for acute anterior circulation stroke : a meta-analysis of observational studies. **J Korean Neurosurg Soc 61 :** 467-473, 2018
20. Kim HC, Rhim JK, Ahn JH, Park JJ, Moon JU, Hong EP, et al. : Machine learning application for rupture risk assessment in small-sized intracranial aneurysm. **J Clin Med 8 :** 683, 2019.
21. Komotar RJ, Schmidt JM, Starke RM, Claassen J, Wartenberg KE, Lee K, et al. : Resuscitation and critical care of poor-grade subarachnoid hemorrhage. **Neurosurgery 64 :** 397-410, 2009
22. Li D, Hu L, Liang Q, Zhang C, Shi Y, Wang B, et al. : Peripheral T cell receptor beta immune repertoire is promptly reconstituted after acute myocardial infarction. **J Transl Med 17 :** 40, 2019
23. Marik PE, Flemmer M : The immune response to surgery and trauma: implications for treatment. **J Trauma Acute Care Surg 73 :** 801-808, 2012
24. Mathiesen T, Andersson B, Loftenius A, von Holst H : Increased interleukin-6 levels in cerebrospinal fluid following subarachnoid hemorrhage. **J Neurosurg 78 :** 562-567, 1993
25. Moraes L, Grille S, Morelli P, Mila R, Trias N, Brugnini A, et al. : Immune cells subpopulations in cerebrospinal fluid and peripheral blood of patients with aneurysmal subarachnoid hemorrhage. **Springerplus 4 :** 195, 2015
26. Okazaki T, Kuroda Y : Aneurysmal subarachnoid hemorrhage: intensive care for improving neurological outcome. **J Intensive Care 6 :** 28, 2018
27. Park JJ, Kim BJ, Youn DH, Choi HJ, Jeon JP : A Preliminary Study of the Association between SOX17 gene variants and intracranial aneurysms using exome sequencing. **J Korean Neurosurg Soc 63 :** 559-565, 2020
28. Robins H, Desmarais C, Matthis J, Livingston R, Andriesen J, Reijonen H, et al. : Ultra-sensitive detection of rare T cell clones. **J Immunol Methods 375 :** 14-19, 2012
29. Robins HS, Campregher PV, Srivastava SK, Wacher A, Turtle CJ, Kahsai O, et al. : Comprehensive assessment of T-cell receptor beta-chain diversity in alphabeta T cells. **Blood 114 :** 4099-4107, 2009
30. Sims JS, Grinshpun B, Feng Y, Ung TH, Neira JA, Samanamud JL, et al. : Diversity and divergence of the glioma-infiltrating T-cell receptor repertoire. **Proc Natl Acad Sci U S A 113 :** E3529-E3537, 2016
31. Sun J, Sun B, Gao Y, He F, Yang L, Wang M, et al. : Composition and variation analysis of the T cell receptor β-chain complementarity determining region 3 repertoire in neonatal sepsis. **Scand J Immunol 86 :** 418-423, 2017
32. Yang G, Ou M, Chen H, Guo C, Chen J, Lin H, et al. : Characteristic analysis of TCR β-chain CDR3 repertoire for pre- and post-liver transplantation. **Oncotarget 9 :** 34506-34519, 2018
33. Youn DH, Kim BJ, Kim Y, Jeon JP : Extracellular mitochondrial dysfunction in cerebrospinal fluid of patients with delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage. **Neurocrit Care 33 :** 422-428, 2020
34. Yu J, Shi B, Ma L, Liu C, Sun S, Ma R, et al. : Case report for recurrent and new-onset SLE patients treated by high-dose glucocorticoid therapy: characteristics of peripheral TCR beta chain CDR3 repertoires. **Medicine (Baltimore) 96 :** e9022, 2017