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Original Article

Elevated circulating level of CCR6 is related to acute myocardial infarction and severity of coronary artery stenosis

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Abstract: Aims: The chemokine receptor 6 (CCR6) is involved in the development of atherosclerosis as a novel mediator of inflammation and immune system. However, the relationship between CCR6 and acute myocardial infarction (AMI) is unclear. This study was designed to determine whether the level of circulating CCR6 is increased in Chinese patients with AMI. Methods: Flow cytometry was used to assay the circulating $CCR6^+$ lymphocyte percentage in 44 patients with AMI and 44 controls. Binary logistic regression analysis was used to identify the relationship between CCR6 and AMI. Results: The percentages of $CCR6^+$ lymphocyte in patients with AMI were significantly higher than those of controls (16.1 vs. 8.4%, $P<0.01$). Furthermore, $CCR6^+$ expression level was an independent biomarker of AMI (OR: 4.73, 95% CI: 1.88-11.91, $P<0.01$). The $CCR6^+$ lymphocyte percentage was correlated positively with the severity of coronary artery stenosis as determined by Gensini score in AMI group. Conclusions: This study showed high level of CCR6 expression in AMI and identified CCR6 as a novel independent biomarker for AMI. Our results suggested that CCR6 may work as a mediator of T lymphocyte recruitment in AMI.

Keywords: CCR6, lymphocyte, Gensini score, atherosclerosis, acute myocardial infarction

Introduction

Myocardial infarction is a cardiovascular disease that severely threatens human health and life, and whose primary cause is atherosclerosis of coronary artery. The pathogenesis of atherosclerosis involves the accumulation of lipids and leukocytes in the intima of blood vessel walls creating plaque, inflammation and both innate and adaptive immunities [1, 2]. Accumulating evidence showed that destabilization of this atherosclerotic disease is associated with T lymphocyte activation [3, 4].

The chemokine system, including chemokines and chemokine receptors, is a crucial mediator and regulator of leukocyte trafficking during inflammation and immune response [5]. The CC chemokine receptor 6 (CCR6) is a member of the seven-transmembrane-domain G-protein-coupled receptors. This receptor is expressed prominently on various leukocyte subsets, including immature dendritic cells, B cells, T cells (Th17 and Treg cells), γδ T cells, NKT cells, neutrophils, and monocytes [6-9]. In the studies of experimental autoimmune encephalomyelitis (EAE), researchers found that CCR6 is essential for priming autoreactive $CD4^+$ T cells and it is associated with the overall clinical severity of EAE [10, 11]. In the study of antitopoisomerase-positive systemic sclerosis [12], psoriasiform dermatitis [13], and granulomatosis with polyangiitis [14], researchers found that $CCR6^+$ immune cells are recruited toward to the lesion location through CCR6-CC chemokine ligand 20 (CCL20) axis to participate in regulating immune response. In addition, Wan, et al. [9] found that CCR6 is associated with the area of aortic lesion and promotes atherosclerosis in ApoE-deficient mice. Yilmaz, et al. [15] showed a high expression of CCR6 at the upstream shoulder of human carotid artery plaque. In conclusion, these studies revealed that CCR6 plays an important role as a mediator of inflammation and immune response in atherosclerosis or autoimmune diseases.

Table 1. Characteristics of AMI patients and controls

	AMI (n=44)	Controls (n=44)	p
Age, means (± SD)	63.8 (± 14.2)	59.2 (± 11.2)	0.176
Male, n (%)	36 (81.8)	31 (70.5)	0.211
Hypertension (%)	24 (54.5)	18 (40.9)	0.20
Diabetes (%)	9 (20.5)	6 (13.6)	0.395
Smoking (%)	16 (36.4)	18 (40.9)	0.661
Laboratory parameters			
TC (mmol/L)	4.91 (4.19-6.44)	4.80 (4.04-5.53)	0.139
TG (mmol/L)	1.49 (1.00-2.14)	1.32 (0.94-2.11)	0.372
LDL (mmol/L)	3.10 (2.62-4.35)	2.63 (1.91-3.28)	0.001*
HDL (mmol/L)	0.93 (0.78-1.10)	1.23 (1.04-1.55)	0.000*
WBC ($\times 10^9$/L)	8.82 (7.39-10.62)	5.99 (4.99-7.61)	0.000*
UA (μmol/L)	362.6 (± 104.3)	362.1 (± 72.5)	0.98
$CCR6^+$ lymphocyte (%)	16.1 (11.9-20.0)	8.4 (5.6-12.9)	0.000*

Data presented as mean ± SD or median (interquartile range) for variables. AMI, acute myocardial infarction; TC, total cholesterol; TG, triglycerides; LDL, low-density lipoprotein; HDL, high-density lipoprotein; WBC, white blood count; UA, uric acid. *P<0.01 AMI vs. control.

However, few studies focused on the CCR6 expression in clinical patients and the relationship between CCR6 and acute myocardial infarction (AMI) has not been established. Therefore, our study is aimed to determine whether the level of circulating CCR6 would increase in Chinese patients with AMI.

Materials and methods

Study subjects

The prospective study was approved by the Medical Ethics Committee of the First Affiliated Hospital of Wenzhou Medical College (China), in accordance with the Declaration of Helsinki. Patients with AMI (ICD-9 codes 410) undergoing coronary angiography in our cardiac care unit were consecutively recruited (**Table 1**). AMI, including ST-segment elevation myocardial infarction and non-ST-segment elevation myocardial infarction, was diagnosed according to the presence of two of these criteria: a. prolonged chest pain; b. typical ECG changes; c. increased troponin I level (>0.15 μg/l). The control group comprised 44 patients recruited from the Cardiology Department. These patients presented chest discomfort and certain risk factors for coronary disease, but there coronary angiography results were normal. Patients in both AMI group and control group were treated with DAPT and statin in a standard manner. Written informed consent was obtained from all subjects before enrollment into this study. Exclusion criteria included patients with ischemic stroke history, peripheral vascular disease, hematological diseases, acute or chronic inflammation, severe liver or renal dysfunction, autoimmune disease, and cancer.

Sample collection

In the AMI group, blood samples were obtained immediately after coronary angiography or percutaneous coronary intervention within 48 h after admission. In the control group, blood samples were obtained immediately after coronary angiography following their admission. Peripheral venous blood was drawn from the antecubital vein while the patients were in a fasting state. Blood was collected using EDTA as an anticoagulant.

Assessment of the severity of coronary artery stenosis

Coronary angiography was performed by using a standard technique. The severity of coronary stenosis in the AMI patients was estimated by the Gensini coronary score following coronary angiography. The Gensini score was calculated by estimating the narrowing of each coronary artery lumen and the significance of the lesion location. The reductions in the lumen diameter of 25%, 50%, 75%, 90%, 99% and complete occlusion were assigned Gensini scores of 1, 2, 4, 8, 16, and 32 respectively. The score was multiplied by a multiplying factor which depended on the following functional significance of the position of the lesion in the coronary arterial: 5 for the left main coronary artery; 2.5 for the proximal left anterior descending coronary artery (LAD) or left circumflex artery (LCX); 1.5 for the mid-LAD; and 1 for the distal LAD, the mid-distal LCX, or the right coronary artery.

Flow cytometric analysis of CCR6

The percentages of $CCR6^+$ lymphocyte were measured by flow cytometry. Approximately 100 μl of blood was added into two tubes separately and incubated with APC antihuman

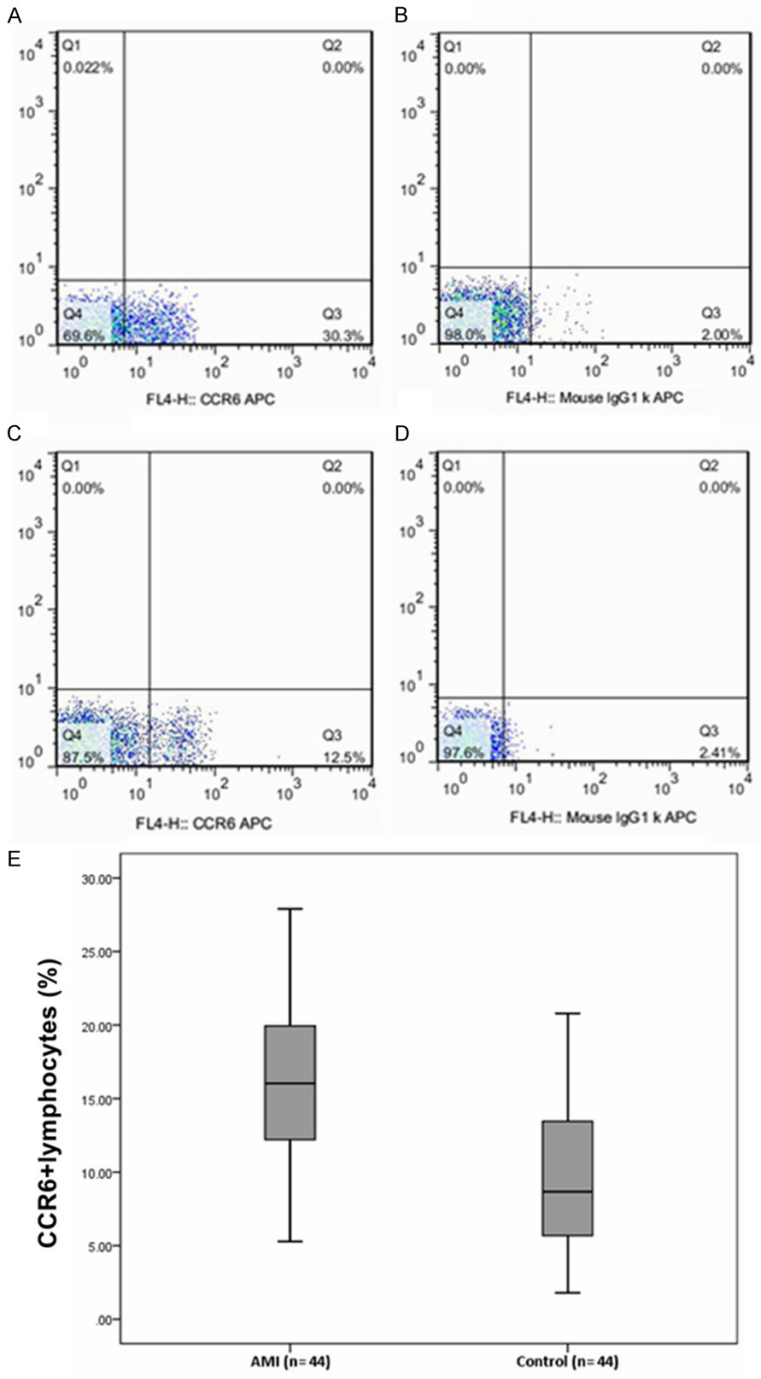

Figure 1. The difference percentages of CCR6$^+$ lymphocytes between AMI group and controls. A and B: Flow cytometry analysis of CCR6 expression in patients with AMI; C and D: Flow cytometry analysis of CCR6 expression in controls; E: Percentages of CCR6$^+$ lymphocytes in patients with acute myocardial infarction (AMI) and controls. Horizontal line in the box plots represents the median value; the boxed area is the interquartile range and the whiskers denote the 2.5th and 97.5th percentiles. *P<0.01 AMI vs. control.

CCR6 antibody (eBioscience, San Diego, CA) and Mouse IgG1 K isotype control APC (Both antibodies are from eBioscience, San Diego, CA) at room temperature for 30 min. After staining, 2 ml of red blood lysing solution was added for FACS (MultiSciences, Hangzhou, CN) into each tube and incubated at room temperature for 10 min. The stained cells were washed once in phosphate-buffered saline (PBS) and suspended in 200 μl of PBS. All flow cytometry was performed using a FACS Calibur (BD Biosciences, USA), and the data were analyzed and presented using Flowjo software.

Statistical analysis

Statistical analysis was performed with the SPSS v.19 statistical software (SPSS Inc., Chicago, IL) and Stata v.14 statistical software. All of the data were described as mean ± SD or median (interquartile range). Independent-samples T test or Wilcoxon rank sum test was used for comparisons of two groups of individuals. Chi-square test was used to compare proportions. Pearson correlation coefficient was used to assess the relationship between the percentages of CCR6$^+$ lymphocyte and Gensini score. Binary logistic regression analysis was used to identify the important risk factors. In all tests a value of $P<0.05$ was considered statistically significant.

Results

Clinical characteristics of patients

The characteristics of patients with AMI and those of controls are shown in **Table 1**, the original datas of each individual participants are shown in Supplementary Table 1. No statistical differences were observed in age, male, hypertension, diabetes, smoking, total cholesterol, triglycerides and uric acid between two groups, but several interrelated factors

Table 2. Binary logistic regression analysis for acute myocardial infarction

Factors	Univariate (n=88)					Multivariate (n=88)				
	β	OR	95% CI		*p*	β	OR	95% CI		*p*
			Low	Upper				Low	Upper	
Age	0.03	1.03	1.00	1.06	0.10	0.07	1.08	1.00	1.16	0.058
Hypertension*	0.55	1.73	0.75	4.03	0.20	0.58	1.78	0.26	12.06	0.553
Diabetes*	0.49	1.63	0.53	5.04	0.398	0.63	1.88	0.17	20.35	0.605
Smoking*	-0.19	0.83	0.35	1.95	0.66	0.32	1.37	0.24	7.75	0.72
LDL	0.80	2.22	1.35	3.66	<0.01	1.67	5.31	1.56	18.09	0.008
HDL	-3.75	0.024	0.004	0.16	<0.01	-3.67	0.03	0.00	0.58	0.021
WBC	0.53	1.71	1.32	2.20	<0.01	0.70	2.00	1.28	3.15	0.003
$CCR6^+$ lymphocyte quartile#	1.02	2.77	1.73	4.46	<0.01	1.36	3.91	1.83	8.35	0.000

LDL, low-density lipoprotein; HDL, high-density lipoprotein; WBC, white blood count. *patients without hypertension, diabetes and smoking were set as the reference group respectively. #patients with the lowest quartile of $CCR6^+$ lymphocyte percentage were set as the reference group.

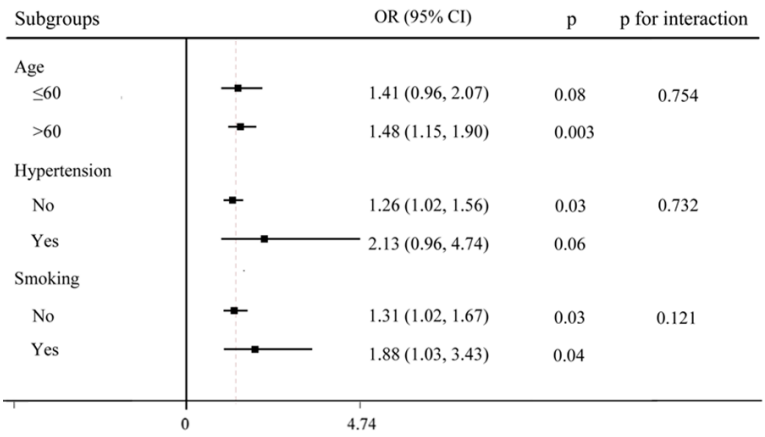

Figure 2. Odds Ratios for AMI in specified subgroups.

such as low-density lipoprotein (LDL) and white blood count (WBC), were higher in AMI group than those in the control group (*P*<0.01). On the contrary, high-density lipoprotein (HDL) was lower in AMI group (*P*<0.01) compared with that in the controls.

$CCR6^+$ lymphocyte percentage was elevated in patients with AMI

The $CCR6^+$ lymphocyte percentage measured by flow cytometry analysis is shown in **Figure 1**. The percentages of $CCR6^+$ lymphocyte in patients with AMI were significantly higher than those of controls (16.1% vs. 8.4%, *P*<0.01, **Figure 1**).

$CCR6^+$ lymphocyte level was an independent biomarker of AMI

In binary logistic regression analysis, LDL, HDL, WBC, and $CCR6^+$ lymphocyte quartiles were associated with AMI by univariate analysis (**Table 2**). LDL and WBC were independent factors of AMI in the multivariate analysis. (OR: 5.31, 95% CI: 1.56-18.09, *P*<0.01; OR: 2.00, 95% CI: 1.28-3.15, *P*< 0.01). HDL was an independent protective factor of AMI (OR: 0.03, 95% CI: 0.00-0.58, *P*=0.021). Furthermore, $CCR6^+$ lymphocyte quartile was a novel independent factor of AMI. (OR: 3.91, 95% CI: 1.83-8.35, *P*<0.01). Stratified analyses were performed by age, hypertension and cigarette smoking status. There were no significant interactions in any of the subgroups (*P*>0.05 for all comparisons) (**Figure 2**).

$CCR6^+$ lymphocyte percentage was correlated positively with the severity of coronary artery stenosis

A significant Pearson correlation coefficient was noted between angiographic Gensini score and circulating level of $CCR6^+$ lymphocyte (R^2=0.517, *P*<0.01) (**Figure 3**).

Discussion

In the present study, we first indicated the high circulating level of CCR6 in the AMI patients and showed that CCR6 expression level is an independent biomarker of AMI. CCR6, which is the sole receptor for the CCL20 [16], possesses both inflammatory and homeostatic functions [5]. One previous study observed no sig-

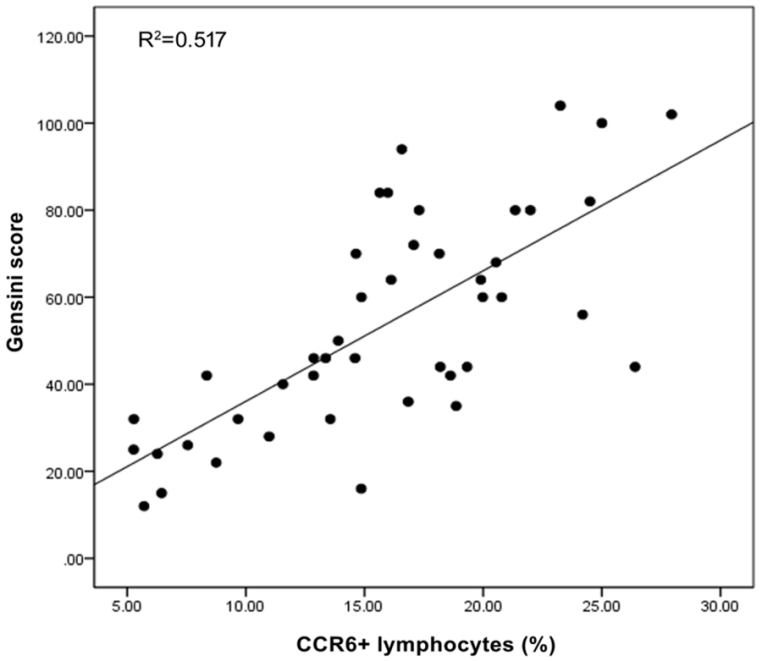

Figure 3. Relationship between the percentages of $CCR6^+$ lymphocyte and coronary angiographic. Gensini score in AMI group. (n=44, R^2=0.517, *P*<0.01).

nificant modulation of both Th17 and Treg cells in hypercholesterolaemia $CCR6^{-/-}Idlr^{-/-}$ mice [17]. However, early study found that the aortic lesion area in $CCR6^{-/-}ApoE^{-/-}$ mice is 30%-40% smaller than that in $CCR6^{+/+}ApoE^{-/-}$ mice; additionally, the macrophage content of lesions in $CCR6^{-/-}ApoE^{-/-}$ mice shows 44% less lesions than those in $CCR6^{+/+}ApoE^{-/-}$ mice, which indicated that CCR6 promotes atherosclerosis in ApoE-deficient mice [9]. Moreover, Yan, et al. [18] suggested that CCL20-CCR6 signaling pathway mediates the migration of γδ T cells into infarcted heart. Consistent with these results, our study further showed that the circulating $CCR6^+$ lymphocyte frequency increases in Chinese patients with AMI. These data indicated CCR6 level, as a molecular marker, is closely associated with the inflammation and immune response in the pathogenesis of AMI.

To our knowledge, coronary lesions infiltrated with T cells are prone to be remarkably unstable. Several clinical studies demonstrated that the activation levels of circulating T lymphocytes increase in patients with unstable angina [19, 20]. Furthermore, peripheral circulating T lymphocytes change during the development of atherosclerotic disease [21, 22]. On the basis of these findings, we hypothesized that CCR6 may play a significant role in recruiting certain subsets of T lymphocytes in peripheral blood of patients with AMI. This receptor may also be closely related with the destabilization of atherosclerotic plaque.

This study also indicated that CCR6 expression level is a novel independent biomarker for AMI. This association was maintained after adjusting for other acknowledged independent risk factors such as age, hypertension, diabetes, smoking, and LDL. In addition, the percentage of $CCR6^+$ lymphocytes was correlated positively with the severity of coronary artery stenosis as determined by Gensini score in AMI group. This result suggested that CCR6 participates in the development of atherosclerosis.

Nevertheless, this study also presented several limitations. Firstly, the number of Chinese patients with AMI involved in our study is limited, so future study with a large cohort will be required. Secondly, this study was designed as a cross-sectional study that unable to demonstrate causality between CCR6 level and AMI, so further studies will be needed to prove the causality between CCR6 expression and atherosclerosis development.

In summary, our study showed high $CCR6^+$ lymphocyte percentage in AMI patients and identified this chemokine receptor as an independent biomarker for AMI. We suggest that CCR6 may function as a mediator of recruiting T lymphocytes which are involved in immune response and associated with the destabilization of coronary lesions.

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Disclosure of conflict of interest

None.

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Supplementary Table 1. The original datas of each individual participants

No.	Group (1=AMI; 2=control)	Age	Sex (male=1; female=2)	Hypertension	Diabetes	Smoking	WBC	TC	LDL	HDL	TG	UA	CCR6 percentage	CCR6 quartiles	Gensini score
1	1	62	1	1	0	0	8.58	5.86	3.86	1.13	2.1	292	6.27	1	24
2	1	46	1	0	1	0	5.86	4.34	2.79	1.16	0.32	389	8.35	2	42
3	1	73	1	0	1	0	7.73	4.93	3.09	0.92	1.68	233	17.3	4	80
4	1	65	1	0	0	1	11.76	4.53	2.93	0.9	0.98	233	8.75	2	22
5	1	58	1	1	0	0	10.43	2.83	1.35	0.61	1.89	383	13.36	3	46
6	1	61	1	0	0	1	7.27	4.35	2.95	0.8	1.55	462	18.86	4	35
7	1	81	1	1	0	1	8.21	3.97	2.15	1.04	1.05	408	24.5	4	82
8	1	35	1	0	0	0	15.68	7.39	6	0.71	1.26	377	16.12	3	64
9	1	40	1	0	0	0	16.98	7.94	5.72	0.93	2.64	313	16.84	3	36
10	1	79	1	1	0	0	7.61	4.1	2.35	1.02	1.23	471	6.45	1	15
11	1	51	1	0	0	0	9.39	4.53	2.57	0.85	2.45	249	20.78	4	60
12	1	77	1	0	0	0	9.96	5.7	3.8	1.07	0.75	489	14.87	3	60
13	1	55	1	0	1	1	8.27	4.89	2.73	1.18	2.4	292	18.15	4	70
14	1	85	2	1	0	0	7.99	7.41	5.06	1	2.21	527	10.98	2	28
15	1	40	1	0	0	0	15	6.87	5	0.91	2.14	257	26.4	4	44
16	1	47	1	0	0	1	8.65	6.54	4.76	1.11	1.17	295	24.19	4	56
17	1	69	1	1	0	1	7.82	3.72	1.8	0.99	1.83	327	18.19	4	44
18	1	73	1	0	0	1	5.33	5.44	4.05	0.78	0.62	529	23.25	4	104
19	1	58	1	1	0	1	9.06	8.12	6.09	0.79	2.27	431	27.94	4	102
20	1	69	1	0	1	1	6.39	4.77	3.21	1.21	0.99	200	25	4	100
21	1	44	2	0	0	0	3.92	7.95	5.98	0.77	2.21	228	14.86	3	16
22	1	61	1	1	0	0	9.75	4.85	3.34	0.92	1.69	352	13.56	3	32
23	1	70	2	0	0	0	7.18	3.85	2.86	0.65	1.55	443	21.35	4	80
24	1	62	1	1	1	0	8.67	7.19	4.94	0.67	2.84	664	13.89	3	50
25	1	72	1	1	0	0	8.96	4.26	2.56	0.99	0.9	440	11.56	2	40
26	1	64	1	1	1	0	13.96	3.64	2.58	0.61	0.87	586	5.27	1	25
27	1	76	1	1	0	0	10.98	4.16	2.82	0.78	1.1	297	5.28	1	32
28	1	72	1	0	0	1	6.34	5.01	2.78	1.44	1.46	374	5.71	1	12
29	1	85	1	0	0	0	5.41	4.53	2.93	0.99	1.28	372	12.86	3	46
30	1	55	1	0	0	0	11.64	6.47	4.48	0.81	2.63	263	19.32	4	44
31	1	63	1	1	0	0	9.34	5.15	3.74	0.71	1.03	352	19.98	4	60
32	1	50	1	1	0	1	8.98	6.35	4.09	0.94	1.78	411	7.55	2	26
33	1	77	1	1	0	0	7.48	3.79	1.66	0.67	3.59	306	14.64	3	70

34	1	82	2	1	1	0	7.36	7.14	4.89	0.93	2.12	198	9.67	2	32
35	1	30	1	1	1	1	11.2	5.18	3.07	0.93	2.27	331	20.54	4	68
36	1	72	2	1	0	0	9.52	6.48	4.44	1.44	0.29	230	18.62	4	42
37	1	67	2	1	0	0	9.74	4.07	2	1.22	1.2	335	21.99	4	80
38	1	73	2	1	0	0	6.01	5.54	3.35	1.45	1.42	324	19.9	4	64
39	1	66	2	1	1	0	16.12	5.17	3.11	0	1.7		15.98	3	84
40	1	86	1	1	0	1	10.68	4.56	2.44	1.36	0.89	429	16.57	3	94
41	1	81	1	1	0	1	5.53	4.1	2.83	0.81	0.75	357	14.6	3	46
42	1	65	1	0	0	1	9.88	3.44	1.94	0.95	1.01	354	17.07	4	72
43	1	44	1	1	0	0	11.5	5.48	3.66	1.07	1.52	457	15.64	3	84
44	1	66	1	0	0	1	8.19	4.81	3.4	1.3	0.36	333	12.85	2	42
45	2	62	1	0	0	1	5.82	5.02	2.61	1.69	1.38	235	8.7	2	
46	2	63	1	0	0	1	5.77	4.8	2.62	1.39	1.75	379	10.5	2	
47	2	55	1	1	0	1	7.29	3.56	1.74	1.3	0.83	475	11.2	2	
48	2	77	1	0	1	0	6.54	4	2.31	0.95	1.67	435	13.14	3	
49	2	40	1	0	0	0	5.64	4.64	2.82	0.97	2.11	320	5.7	1	
50	2	44	1	0	0	0	7.03	6.15	3.04	1.98	2.1	384	18.7	4	
51	2	43	1	1	0	0	4.22	4.29	2.6	1.12	1.15	499	11.5	2	
52	2	70	1	0	0	0	6.05	5.79	3.36	1.44	2.49	404	16.9	4	
53	2	44	1	1	0	0	5.01	4.77	1.79	1	5.51	514	14.6	3	
54	2	69	1	0	0	1	4.73	3.95	1.63	1.51	2.46	352	4.71	1	
55	2	49	1	0	0	1	7.63	3.68	1.88	1.26	0.99	300	8.1	2	
56	2	54	1	0	0	1	8.14	4.15	2.34	1.19	0.89	383	13.5	3	
57	2	50	1	0	0	0	6.27	5.25	2.46	2.3	0.7	267	13	3	
58	2	49	1	0	0	1	3.82	6.99	4.46	1.56	1.33	367	14.15	3	
59	2	48	1	1	0	1	4.28	5.48	3.86	1.21	0.76	355	5.55	1	
60	2	59	1	0	1	1	8.29	6.23	2.69	1.14	6.44	394	5.64	1	
61	2	67	1	0	0	1	5.77	5.54	3.21	1.47	1.1	447	9.89	2	
62	2	71	1	0	0	1	6.11	4.51	2.78	1.12	1.31	363	10	2	
63	2	61	2	0	0	1	10.49	6.22	3.3	1.28	2.48	479	3.24	1	
64	2	42	1	1	0	0	8.89	5.5	2.76	0.99	4.41	378	4.13	1	
65	2	62	1	1	0	0	6.1	4.42	2.3	0.82	2.97	466	6.76	2	
66	2	77	1	0	0	0	5.94	4.96	2.87	1.14	1.49	438	5.74	1	
67	2	43	1	0	0	1	9.17	5.66	3.57	1.13	1.99	375	5.14	1	
68	2	64	1	1	0	1	6.72	4.88	2.24	2	0.77	441	8.75	2	
69	2	51	2	0	0	0	4.59	6.78	3.52	2.28	0.56	376	9.4	2	

70	2	48	2	0	0	0	5.07	4.85	1.85	2.22	0.73	266	12.21	2
71	2	59	1	0	0	0	5.93	5.9	3.72	1.68	0.65	295	20.8	4
72	2	57	2	0	0	0	4.98	3.42	1.41	1.63	0.37	318	6.98	2
73	2	59	1	0	0	1	10.36	3.22	1.82	0.85	1.21	247	2.02	1
74	2	69	1	1	0	1	10.1	4.29	2.64	1.25	0.93	333	1.44	1
75	2	85	1	1	0	1	9.22	3.54	2.14	0.76	1.19	298	1.29	1
76	2	59	2	0	1	0	7.62	4.25	2.38	1.04	1.44	359	5.55	1
77	2	47	2	0	0	0	7.58	5.22	2.86	1.68	0.73	234	4.42	1
78	2	67	2	1	0	0	5.23	6.1	3.85	1.08	1.31	354	3.22	1
79	2	66	1	0	0	1	5.1	4.91	2.94	1.29	1.06	326	3.58	1
80	2	70	1	1	1	0	4.72	3.11	1.77	1.04	0.97	311	6.39	1
81	2	52	2	0	1	0	6.7	5.08	3.39	1.16	1.72	351	12.87	3
82	2	74	2	1	0	0	4.21	3.93	1.77	1.39	0.96	325	16.13	3
83	2	69	2	1	0	0	5.67	4.65	1.99	0.8	4.2	458	12.9	3
84	2	48	1	1	1	0	4.17	3.79	1.55	1.05	2.78	383	7.57	2
85	2	64	1	1	0	0	4.67	4.51	3.38	0.65	1.57	235	7.61	2
86	2	77	2	1	0	0	10.57	4.79	2.98	1.41	0.99	330	6.32	1
87	2	60	2	1	0	0	4.73	7.98	4.41	1.75	2.06	302	12.59	2
88	2	62	2	1	0	0	6.04	3.52	1.87	0.78	2.3	383	12.86	3