Acquired Immuno-deficiencies along in Cardio Vascular Disease-Special Reference to Heparinized System

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Abstract

Background: The purpose of this report was to introduce the one of typical acquired immunodeficiency (AID) about an immunological factors during systemic surgical operation in a patient CAPG. AID might serious to connect many infectious diseases. In this article, we would like to show the immunological factors along with quantitative aspects around ECC operation, together with symptom for monitoring therapeutic counselling.

Methods: The blood sample were collected from CABG patients, on and off of CABG, 1d, 3d, 7d, 14d, totally 7 times for assay. Another, age matched normal controls were recruited by the issue for colon cancer patient. The monitoring tests were set up for CD2$^+$, CD4$^+$, CD8$^+$, CD11b$^+$, CD16$^+$, CD19$^+$ and CD57$^+$ positive cells were followed up to 14 days after the operation. The trials were monitored adjusting the time zone so as to coincide the circadian rhythm of leukocyte. The monitoring tests were set up for CD2$^+$, CD4$^+$, CD8$^+$, CD11b$^+$, CD16$^+$, CD19$^+$ and CD57$^+$ positive cells were followed up to on and off of CABG, 1d, 3d, 7d, 14 day after the operation. The trials were monitored adjusting the time zone so as to coincide the circadian rhythm of leukocyte.

Results: The quantitative regulation of total leucocyte number was not seen in both pre and post ECC operation. CD2$^+$, CD4$^+$, CD8$^+$, CD11b$^+$, CD16$^+$, CD19$^+$ and CD57$^+$ lymphocytes were lineup in this report. The dynamics of CD2$^+$, CD4$^+$, CD8$^+$, CD11b$^+$, CD16$^+$, CD19$^+$ and CD57$^+$ lymphocytes, significant decrease were evident down regulated 1d, 3d, 7d and 14 days ($P < 0.05$). However, in 14 days, the numbers got pretty recovery post CAPG.

Conclusion:

1) Total leucocyte was decreased 60 min after CAPG, but recovered 1d, 3d, 7d and 14 days after ECC ($P < 0.05$).
2) Total lymphocyte numbers were not different around CAPG. However, down regulated 1d, 3d, 7d and 14days ($P < 0.05$).
3) CD positive cell was down regulated 1d, 3d, 7d and 14 days after operation.
4) All the CD positive cells were significantly affected by around CAPG operation.
5) There was no significant difference in immunological factors between NH and HG group.

Keywords: ECC; CABG; AID; CD2$^+$; CD4$^+$; CD8$^+$; CD11b$^+$; CD16$^+$; CD19$^+$; CD57$^+$

Abbreviations

AMI: Acute Myocardial Infarction; AID: Acquired Immune Deficiency; ECC: Extracorporeal Circulation; CABG: Coronary Artery Bypass Grafting; CD2: Lymphocyte Subset Marker Possible to Differentiate Further Mature T-Cell; CD4: Helper/Inducer T-Cell Marker; CD8: Regulative/Delayed Type Hypersensitive T-Cell Marker; CD16: Cell Surface Marker on the NK Cell Marker; CD19: B-Cell Marker; CD57: NK Cell Maker; FCM: Flow Cytometry; FITC: Fluorescent Isocyanate Conjugate; HG: Heparin Group; NG: Non-Heparin Group

Introduction

According to the reports from Ministry of Health in each country, the average life span is elongated over the past decade. This trend is expected to be keeping constant further in the future and at the same time continued efforts should be made.

The major factor(s) to regulate is(are) systematic immunological factors, especially defense immunity against external invasive organisms. A vertebrate animal acquired two defense systems ontogenically and phylogenically. Despite these defense systems, overwhelming problems of possessing these dual systems, either innate and adoptive does not seem to guard or even prevent the development of one internal threat to survival. We have been trying to regulate the immune responsiveness through much mature for fragile in daily stress and so on [1]. In this article, we would like to show the regulatory mechanism to the acquired immune-deficient status (AIS) derived from cardio-vascular surgical operation, ECC . The circumstance, except for cases of contraindication, has been medically useful approved to be effective in many stress-related disorders and the improvement of dysfunction of the biological rhythm disturbance as well as chronic disease. The mechanism of effects has been reported in many studies, but many things are still unclear. We had prepared the native non-specific gourd line outside of the skin and/or mucous membrane. However, the gourd line is easily broken by accidental affair. However, nonspecific and specific attack system had been prepare as lympho-reticular line of defense [2]. So this report was focused on mainly quantitative aspect of immunological factors around ECC, especially in CAPB [3].

Many systems are in place to evaluate Western therapies that aim at healing the symptoms of an illness. However, when the purpose of a therapy is to regulate the immunological factors patient as well as normal one, such as some alternative medical therapies, no widely-accepted evaluation system has been established. To fill this lack, we would like to propose the number and functions of leukocyte subsets as indicators for the evaluation of immune deficient status.

Subjects and Methods

Subjects

Patient and Control

A total of 45 volunteers were enrolled in this study including 15 control group. Thirty patients were recruited from ECC patients and divide in two group, non-heparin(NH) and heparin group(HG), aged 67.5±9.3 and 62.0±8.5, respectively. Sexual ratio were NH and HG, M:13, F:2 and M:9, F6, respectively. All of the patients met the predefined diagnostic criteria and selected by the issue ECCA. Total minute for CPA were NH,124.1±25.2 second, HG,100.4±31.56, respectively. The trials were monitored before starting anti-cancer chemotherapy, adjusting the time zone so as to coincide the circadian rhythm of leukocyte.

The attendant were divided in to two group and each group was started after informed consented. We collected peripheral blood from fore arm vein of them before and after the operation, at the same time zone on the day, in adjusting of circadian rhythm of leukocyte and CD positive lymphocyte [4,5]. We asked and charged on the laboratory of Ishikawa Prefecture Preventive Medicine Association for authorized and precise and reliable assessment. The total and differential leukocyte counts were measured by the automated hematology analyzer XE-2100 (Sysmex, Inc., Kobe, Japan).

Assessment of CD Positive Cells

The analysis of CD positive cells by FCM was measured by gating in the lymphocytes region on the scattered gram.

In order to estimate a CD+ cell, the blood was collected from the subjects by blood test tube containing an anticoagulant EDTA-2K. 100μl of whole blood were mixed with each corresponding antibody. In order to estimate a CD+ cell, the whole blood was collected from the attendants by blood collection tube containing an anticoagulant EDTA-2K. 100μl of whole blood were mixed with each corresponding antibody. Nonspecific reaction of the PE fluorescence was found in the isotype control. Therefore, the real values of the AR expressing cell counts were calculated by subtracting the control values [6]. The CD19+ cells were observed nonspecific reaction which seems to response of the second antibody. However, the significant variation was not seen from the comparison of β2-AR expressing cells before and

after hot-spring hydrotherapy. The mean % of β2-AR expressing cells in the lymphocyte subsets was 18-19% in CD2+ cells, 5% in CD4+ cells, 57% - 63% in CD8+ cells, and 93% - 95% in CD57+ cells. That in CD19+ cells was approximately 100% (data not shown), but we were not able to be confirmed because it was very likely to be the nonspecific reaction. After incubating for 0.5 hr at 4 °C, these samples were hemolyzed abundant RBC with a 10-times dilution FACS Lysing Solution (Becton Dickinson).

Test style of heparin-coating effect

Comparative study was setting up to the same CABG system for coating the hepaline molecule inside the channel. The same immunological factors were traced up for the same schedule except that a coating the same substance, hepalin [6,7].

Statistical Analysis

The statistical analysis along with the groups (before and after trial) for the test of significant difference were calculated by paired t-test and wilcoxon signed-ranks test. As for the examination of the correlation was found a spearman's correlation coefficient by rank test. Data are expressed as means ± standard error of mean (SE). A *P* value < 0.05 was recorded to be statistically significant. The Kendall tau Rank Correlation and the two-sided p-value were also analyzed.

Results

Quantitative Aspect of Total Leukocytes

The attendant were divided in to two group and each group was started after informed consented according to The Ethics Committee of Kanazawa Medical University. The blood sample were prepared at the same time zone of the first sampling.

The number of lymphocyte were about the same number between normal volunteer and patient before operation, $21.6 \pm 4.3 \times 10^2$/ and $23.9 \pm 4.7 \times 10^2$/.

However, the number of lymphocyte were clearly decreased 2 weeks after operation , $14.2 \pm 3.2 \times 10^2$/ (P<0.001).

Then the number gradually recover to the normal value but still significantly lower than that of normal even both in 4 weeks and 12 weeks ((P<0.05).

Leukocyte Count						
	Before ECC	**After 60` ECC**	**1d**	**3d**	**7d**	**14d**
No.1	3828	2417	2759	2541	3207	3774
No.2	6920	4369	4988	5104	7086	7505
No.3	2503	1422	1624	1495	1699	1799
No.4	5742	3988	4553	5125	7115	7536
No.5	3975	2259	2865	2932	3331	4311
No.6	6110	4244	5921	6059	8412	10889
No.7	5080	3528	4922	5037	5722	6733
No.8	5816	3672	4192	4290	4873	5161
No.9	2429	1534	1946	1991	2513	2661
No.10	2798	1766	2016	2270	2578	2731
No.11	6920	4806	5486	5053	5740	6755
No.12	4712	2975	3396	3823	4342	4599
No.13	5595	3886	5422	5548	7002	7416
No.14	6258	3556	4961	4569	5767	6108
No.15	5006	3161	3608	4062	5126	6033
Lymphocyte Count						
	Before ECC	**After 60` ECC**	**1d**	**3d**	**7d**	**14d**
No.1	18.6	13.5	17	16.7	17.1	17.1

No.2	27.7	20.1	25.4	30.5	34.6	38.6
No.3	27	19.5	24.7	29.7	33.7	37.5
No.4	17.5	15.5	19.6	21.4	21.9	21.9
No.5	25.5	18.5	28.6	34.3	39	47.7
No.6	19	15.3	19.3	19	19.4	23.7
No.7	12.8	10.3	13	15.6	15.9	19.5
No.8	28.4	22.9	32.2	35.1	35.9	36
No.9	30.3	24.4	34.2	33.6	42	51.5
No.10	27.7	24.5	34.5	37.6	47	47.1
No.11	24.8	18	27.8	30.3	34.4	34.5
No.12	35	31	47.9	52.3	53.4	65.5
No.13	31	24.9	31.5	37.9	43	47.9
No.14	14.9	12	18.6	20.3	25.4	25.4
No.15	24.8	20	25.2	27.5	31.3	38.3

***Table 1:** Leukocyte and lymphocyte assessment.*

Blood biochemical parameters

There were no interaction effects between treatment and sampling time for plasma metabolites and humoral immunity ($P > 0.05$) among surgical operation. However, inclusion of operation significantly decreased ($P = 0.05$) concentrations of plasma CRP, C-reactive protein of patient comparted by the control group. Also, there was a time effect on LDL-CH as plasma concentrations decreased with time and significantly so on days 30 and 45 ($P = 0.017$) of the feeding period. Metabolite concentrations of TP, ALB, GLB, BUN, GLU, TG and HDL-CH, and humoral immune indicators, IgG, IgM, and IgA were not affected ($P > 0.05$) by surgical operation itself.

Quantitative aspect of Total Lymphocyte

The number of lymphocyte were about the same number before and after ECC normal volunteer and patient before operation, $21.6\pm4.3\times10^2$/ and $23.9\pm4.7\times10^2$/. However, the number of lymphocyte were clearly decreased 1d, 3d, 7d and 14d after operation (P<0.05).
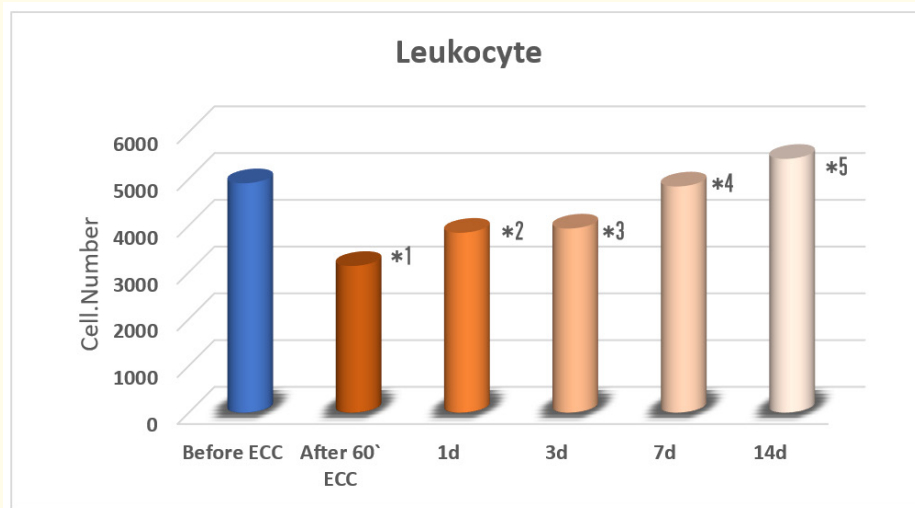

***Figure 1:** Analysis of the Total Leukocyte*
*The blood withdrawn from the patients and normal volunteer by blood collection tube containing an anticoagulant EDTA-2K. 100µl of whole blood were added the anti β2-AR antibody (Santa Cruz Biotechnology, Inc. U.S.A.) of the primary antibody and were reacted for 30 minutes at 4 ⁰C. M In order to estimate a CD+ cell, the blood was collected from the subjects by blood test tube containing an anticoagulant EDTA-2K. 100µl of whole blood were mixed with each corresponding antibody. In order to estimate a CD+ cell, the whole blood was collected from the attendants by blood collection tube containing an anticoagulant EDTA-2K. 100µl of whole blood were mixed with each corresponding antibody. After washing out excessive antibody with PBS, the suspensions were mixed with phycoerythrin (PE)-conjugated streptavidin (Beckman Coulter Inc. France) and fluorescence-activated monoclonal ABs: peridinin chlorophyll protein-cyanin 5.5 (PerCP-Cy5.5)+CD2, fluorescein isothiocyanate (FITC)+CD4, (FITC)+CD8, FITC+CD11b, FITC+CD16, FITC+CD57 (Becton Dickinson Co. U.S.A.), allo-phycocyanin (APC)+CD8, and APC+CD57 (Beckman Coulter). After washing out with Phosphate Buffered Saline, the cell suspensions were fixed employing a X10 diluted Cell FIX (Becton Dickinson) and analyzed by flow cytometer system, FACS Caliber (Becton Dickinson). The negative controls were prepared PE+streptavidin and the isotype control antibodies to the CD antibodies. After incubating for 0.5 hr at 4 ⁰C, these samples were hemolyzed abundant RBC with a 10-times dilution FACS Lysing Solution (Becton Dickinson). A P value < 0.05 was recorded to be statistically significant,*1(P<0.05),
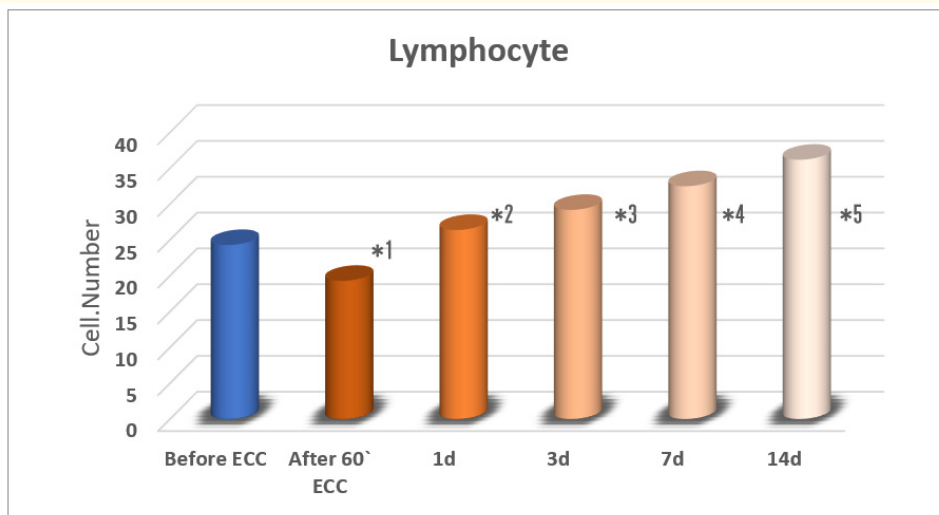*2(P<0.05), *3<0.05).*

Figure 2: *Analysis of the Total Lymphocyte*
The blood withdrawn from the patients and normal volunteer by blood collection tube containing an anticoagulant EDTA-2K. 100μl of whole blood were added the anti β2-AR antibody (Santa Cruz Biotechnology, Inc. U.S.A.) of the primary antibody and were reacted for 30 minutes at 4 ^{0}C. M In order to estimate a CD+ cell, the blood was collected from the subjects by blood test tube containing an anticoagulant EDTA-2K. 100μl of whole blood were mixed with each corresponding antibody. In order to estimate a CD+ cell, the whole blood was collected from the attendants by blood collection tube containing an anticoagulant EDTA-2K. A P value < 0.05 was recorded to be statistically significant,*1(P<0.05), *2(P<0.05), *3<0.05), *4<(P 0.05), *5<(P 0.05).

CD2+

The attendant were divided in to two group and each group was started after informed consented according to the Ethics Committee of Kanazawa Medical University. The blood sample were prepared at the same time zone of the first sampling. The dynamics of CD2+ lymphocyte, multi-potential T-cell subsets, No significant different was seen in quantitative aspect before and after ECC. However, significant decrease were seen after 1d, 3d, 7d and 14 days after operation. The consideration about heparin-coating effect in ECC system, no significant difference was seen between the group HG and NH, Figure 3.

CD2							
	Before ECC	**After 60` ECC**	**Off ECC**	**1d-PO**	**3d-PO**	**7d-PO**	**14d-PO**
No.1	117	114	112	92	98	87	94
No.2	134	143	141	129	112	110	119
No.3	120	117	127	104	90	89	96
No.4	86	92	90	91	96	95	93
No.5	81	79	86	70	61	54	48
No.6	81	79	86	86	91	99	87
No.7	153	134	146	133	141	125	111
No.8	68	66	65	53	57	61	60
No.9	65	63	68	68	66	59	52
No.10	135	145	128	129	112	121	119
No.11	101	108	96	79	68	67	66
No.12	90	88	87	87	75	74	66
No.13	69	60	54	49	52	51	50
No.14	132	141	140	115	122	108	117
No.15	69	74	73	60	64	56	55
CD4							
	Before ECC	**After 60` ECC**	**Off ECC**	**1d-PO**	**3d-PO**	**7d-PO**	**14d-PO**
No.1	123	118	115	96	104	120	108
No.2	60	52	57	52	51	54	49
No.3	117	102	122	112	110	127	127

No.4	66	57	56	47	42	39	39
No.5	114	89	107	89	79	74	67
No.6	141	135	132	121	132	124	137
No.7	111	106	104	87	77	73	65
No.8	134	116	126	116	115	132	132
No.9	72	63	61	51	51	53	53
No.10	69	60	65	49	53	61	55
No.11	102	98	95	80	71	67	60
No.12	114	99	108	90	89	93	102
No.13	105	101	109	91	90	104	93
No.14	57	55	53	49	48	46	50
No.15	117	102	122	112	99	104	104

CD8							
	Before ECC	**After 60` ECC**	**Off ECC**	**1d-PO**	**3d-PO**	**7d-PO**	**14d-PO**
No.1	132	127	119	114	112	99	102
No.2	69	60	69	59	64	63	59
No.3	110	105	121	127	112	100	113
No.4	143	112	129	123	108	118	133
No.5	138	133	125	119	105	93	96
No.6	104	100	94	98	87	77	87
No.7	119	93	107	92	81	88	91
No.8	131	125	118	101	109	119	110
No.9	117	102	118	101	109	118	134
No.10	68	53	56	58	51	56	63
No.11	68	59	62	53	47	46	47
No.12	95	91	86	90	88	78	88
No.13	66	52	60	57	50	45	46
No.14	81	64	67	70	62	61	63
No.15	65	56	53	45	45	40	45

CD16							
	Before ECC	**After 60` ECC**	**Off ECC**	**1d-PO**	**3d-PO**	**7d-PO**	**14d-PO**
No.1	84	102	99	94	106	99	110
No.2	126	152	134	127	117	109	121
No.3	84	92	99	94	96	99	90
No.4	84	83	89	76	78	80	73
No.5	137	150	162	138	140	145	146
No.6	128	126	123	105	107	111	100
No.7	99	98	86	73	75	63	57
No.8	48	58	51	44	44	42	46
No.9	114	113	121	103	105	89	90
No.10	126	125	134	140	128	108	98
No.11	98	107	94	80	90	76	84

No.12	102	123	121	103	115	108	109
No.13	68	74	80	83	93	88	97
No.14	80	87	94	89	91	94	94
No.15	125	151	133	125	141	145	132
CD19							
	Before ECC	**After 60` ECC**	**Off ECC**	**1d-PO**	**3d-PO**	**7d-PO**	**14d-PO**
No.1	105	84	87	100	93	107	114
No.2	92	89	92	97	111	126	111
No.3	83	81	75	86	99	103	90
No.4	132	106	109	114	119	111	108
No.5	62	55	56	59	55	57	61
No.6	65	63	59	55	57	66	64
No.7	125	100	93	107	111	115	112
No.8	140	136	141	162	169	175	187
No.9	62	49	51	53	50	52	45
No.10	75	67	69	72	75	70	68
No.11	137	121	113	118	135	140	150
No.12	99	88	82	94	98	102	99
No.13	126	123	114	132	137	143	138
No.14	102	91	84	88	101	94	83
No.15	104	101	94	108	124	129	125
CD57							
	Before ECC	**After 60` ECC**	**Off ECC**	**1d-PO**	**3d-PO**	**7d-PO**	**14d-PO**
No.1	128	149	149	109	114	107	112
No.2	107	125	112	100	104	88	83
No.3	131	153	168	149	156	147	139
No.4	99	95	104	93	97	82	77
No.5	111	106	95	70	60	62	65
No.6	104	121	121	108	103	96	91
No.7	80	85	93	75	64	61	63
No.8	105	123	110	98	103	87	82
No.9	134	156	156	126	108	112	105
No.10	110	128	128	104	99	102	107
No.11	99	105	95	77	73	75	71
No.12	54	57	52	46	39	41	43
No.13	110	117	128	93	98	101	95
No.14	75	80	88	64	67	69	65
No.15	59	62	56	50	52	49	46

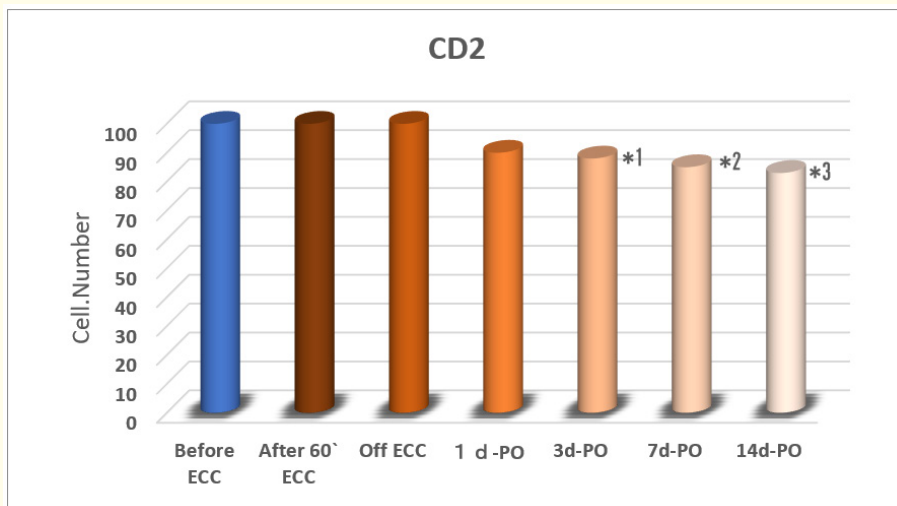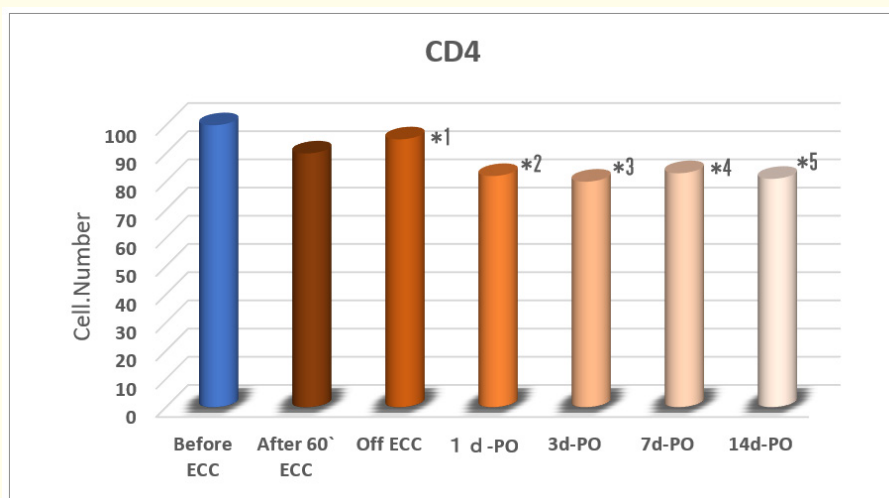***Table 2:*** *CD positive cell count.*

Figure 3: Quantitative aspect of Total Lymphocyte CD2+
*Forty-four patients were enrolled, aged 40~60. All of the patients met the predefined diagnostic criteria and selected Ist and IInd stage in a colon cancer grade. We recruited 15 healthy volunteers in same ager and informed and consented according to The Ethics Committee of Kanazawa Medical University. The trials were monitored before starting anti-cancer chemotherapy, adjusting the time zone so as to coincide the circadian rhythm of leukocyte. In order to estimate a CD+ cell, the whole blood was collected from the attendants by blood collection tube containing an anticoagulant EDTA-2K. 100µl of whole blood were mixed with each corresponding antibody. After washing out excessive antibody with PBS, the suspensions were mixed with phycoerythrin (PE)-conjugated streptavidin (Beckman Coulter Inc. France) and fluorescence-activated monoclonal ABs: peridinin chlorophyll protein-cyanin 5.5 (PerCP-Cy5.5)+CD2, fluorescein isothiocyanate (FITC)+CD2 (Becton Dickinson Co. A P value < 0.05 was recorded to be statistically significant,*1(P<0.05), *2(P<0.05), *3<0.05), *4<(P 0.05), *5<(P 0.05).*

CD4+

The blood sample were prepared at the same time zone of the first sampling. The dynamics of CD4+ lymphocyte, helper/inducer T-cell subsets, significant decrease were seen just 60 minutes after ECC. The consideration about heparin-coating effect in ECC system, no significant difference was seen between the group HG and NH, Figure 4.

Figure 4: Quantitative aspect of Total Lymphocyte CD4+
*Forty-four patients were enrolled, aged 40~60. All of the patients met the predefined diagnostic criteria and selected Ist and IInd stage in a colon cancer grade. We recruited 15 healthy volunteers in same ager and informed and consented according to The Ethics Committee of Kanazawa Medical University. The trials were monitored before starting anti-cancer chemotherapy, adjusting the time zone so as to coincide the circadian rhythm of leukocyte. In order to estimate a CD+ cell, the whole blood was collected from the attendants by blood collection tube containing an anticoagulant EDTA-2K. 100µl of whole blood were mixed with each corresponding antibody. After washing out excessive antibody with PBS, the suspensions were mixed with phycoerythrin (PE)-conjugated streptavidin (Beckman Coulter Inc. France) and fluorescence-activated monoclonal ABs: peridinin chlorophyll protein-cyanin 5.5 (PerCP-Cy5.5)+CD4, fluorescein isothiocyanate (FITC)+CD4 (Becton Dickinson Co. U.S.A.). A P value < 0.05 was recorded to be statistically significant,*1(P<0.05), *2(P<0.05), *3<0.05), *4<(P 0.05), *5<(P 0.05).*

CD8+

The blood sample were prepared at the same time zone of the first sampling. The dynamics of CD8+ lymphocyte, regulative/effector T-cell, significant down regulation were seen at before and after ECC. The down regulation of the group were persisted at 1d, 3d, 7d and 14 days after the operation. The consideration about heparin-coating effect in ECC system, no significant difference was seen between the group HG and NH, Figure 5.
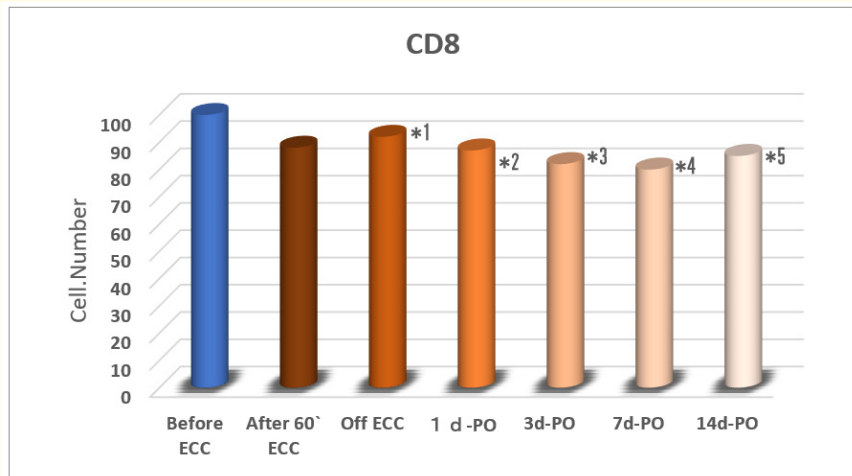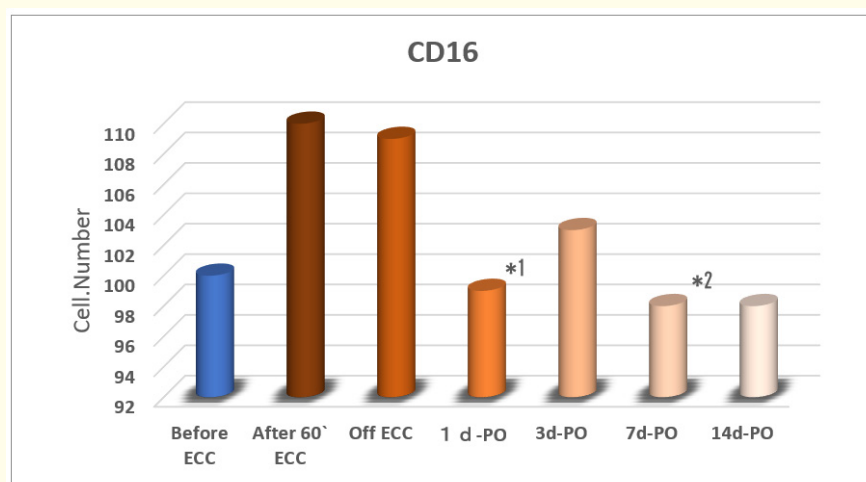

Figure 5: Quantitative aspect of Total Lymphocyte CD8+

Forty-four patients were enrolled, aged 40~60. All of the patients met the predefined diagnostic criteria and selected Ist and IInd stage in a colon cancer grade. We recruited 15 healthy volunteers in same ager and informed and consented according to The Ethics Committee of Kanazawa Medical University. The trials were monitored before starting anti-cancer chemotherapy, adjusting the time zone so as to coincide the circadian rhythm of leukocyte. In order to estimate a CD+ cell, the whole blood was collected from the attendants by blood collection tube containing an anticoagulant EDTA-2K. 100µl of whole blood were mixed with each corresponding antibody. After washing out excessive antibody with PBS, the suspensions were mixed with phycoerythrin (PE)-conjugated streptavidin (Beckman Coulter Inc. France) and fluorescence-activated monoclonal ABs: peridinin chlorophyll protein-cyanin 5.5 (PerCP-Cy5.5)+CD2, fluorescein isothiocyanate (FITC)+CD8 (Becton Dickinson Co. U.S.A.), A P value < 0.05 was recorded to be statistically significant,*1(P<0.05), *2(P<0.05), *3<0.05), *4<(P 0.05), *5<(P 0.05).

CD16+

The blood sample were prepared at the same time zone of the first sampling. The dynamics of CD16+ lymphocyte, along with natural killer cell, significant increases were seen after ECC operation (. The consideration about heparin-coating effect in ECC system, no significant difference was seen between the group HG and NH, Figure 6.
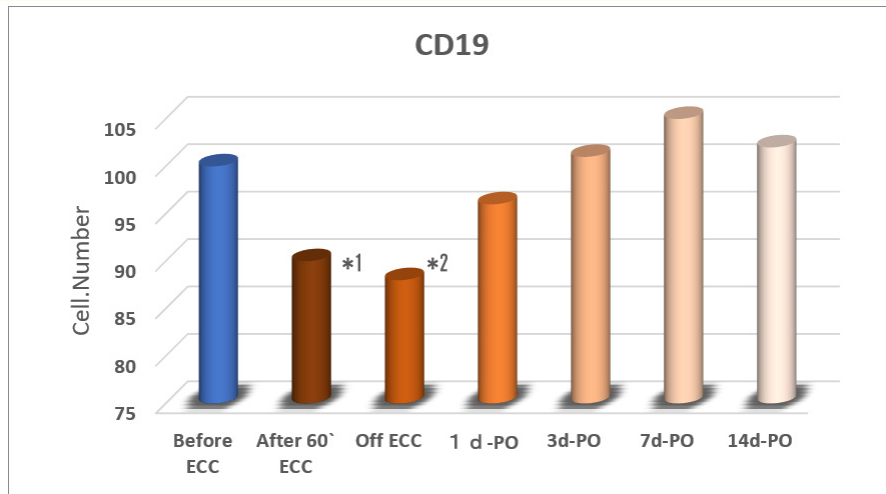

Figure 6: Quantitative aspect of Total Lymphocyte CD16

In order to estimate a CD+ cell, the whole blood was collected from the attendants by blood collection tube containing an anticoagulant EDTA-2K. 100µl of whole blood were mixed with each corresponding antibody. After washing out excessive antibody with PBS, the suspensions were mixed with phycoerythrin (PE)-conjugated streptavidin (Beckman Coulter Inc. France) and fluorescence-activated monoclonal ABs: peridinin chlorophyll protein-cyanin 5.5 (PerCP-Cy5.5)+CD2, fluorescein isothiocyanate FITC+CD11b, (Becton Dickinson Co. U.S.A.), A P value < 0.05 was recorded to be statistically significant,*1(P<0.05), *2(P<0.05).

CD19^{+}

The blood sample were prepared at the same time zone of the first sampling. The dynamics of CD19^{+} lymphocyte, B-cell, significant decrease were seen after ECC operation (P<0.05). The down regulation of the group were persisted at 1d, 3d, 7d and 14 days after the operation. The consideration about heparin-coating effect in ECC system, no significant difference was seen between the group HG and NH, Figure 7.
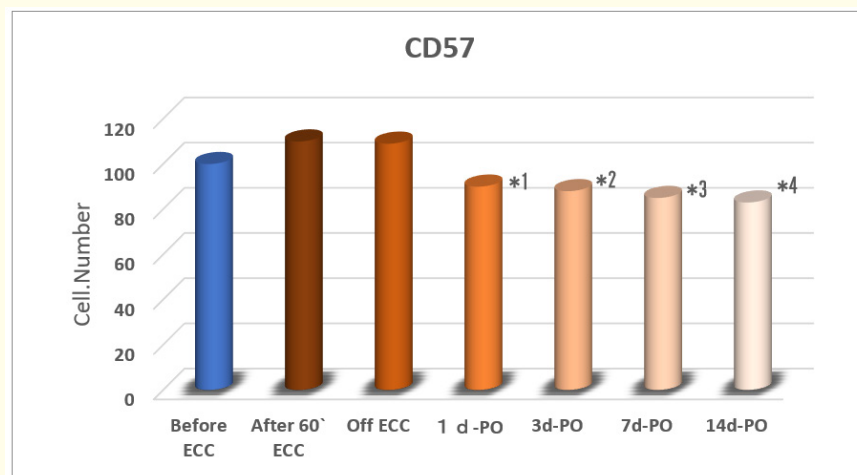

Figure 7: Quantitative aspect of Total Lymphocyte CD19

In order to estimate a CD+ cell, the whole blood was collected from the attendants by blood collection tube containing an anticoagulant EDTA-2K. 100μl of whole blood were mixed with each corresponding antibody. After washing out excessive antibody with PBS, the suspensions were mixed with phycoerythrin (PE)-conjugated streptavidin (Beckman Coulter Inc. France) and fluorescence-activated monoclonal ABs: peridinin chlorophyll protein-cyanin 5.5 (PerCP-Cy5.5)+CD2, fluorescein isothiocyanate FITC+CD16, FITC+CD19 (Becton Dickinson Co. U.S.A.). A P value < 0.05 was recorded to be statistically significant,*1(P<0.05), *2(P<0.05)).

CD57^{+}

The blood sample were prepared at the same time zone of the first sampling. The dynamics of CD57^{+} cell, the marker of natural killer cell along with CD16^{+}, significant increases were seen after ECC operation. The consideration about heparin-coating effect in ECC system, no significant difference was seen between the group HG and NH, Figure 8.

Figure 8: Quantitative aspect of Total Lymphocyte CD57

In order to estimate a CD+ cell, the whole blood was collected from the attendants by blood collection tube containing an anticoagulant EDTA-2K. 100μl of whole blood were mixed with each corresponding antibody. After washing out excessive antibody with PBS, the suspensions were mixed with phycoerythrin (PE)-conjugated streptavidin (Beckman Coulter Inc. France) and fluorescence-activated monoclonal ABs: peridinin chlorophyll protein-cyanin 5.5 (PerCP-Cy5.5)+CD2, fluorescein isothiocyanate FITC+CD57 (Becton Dickinson Co. U.S.A.). A P value < 0.05 was recorded to be statistically significant,*1(P<0.05), *2(P<0.05), *3<0.05), *4<(P 0.05).

Conclusion

In conclusion, the effective assessment of immune-factor alleviated of ECC, as demonstrated by its systemic and anticoagulant properties. In particular, the present study has identified a suitable target for the further scientific investigation of AID. ECC are responsible for the systemdependent immunodeficiency (1). Previous studies have shown that the degradation of the extra matrix that is associated predominantly with collagenous connective tissue may promote pannus hyperplasia, leading to the artificial stress for the circulation, ligaments and heparin are very closely associated with cardiovascular system. The blood sample were collected from ECC patients, on and off of CABG, 1d, 3d, 7d, 14d, totally 7 times for assay. And. Another, age matched normal controls were recruited by the issue for colon cancer patient. The monitoring tests were set up for CD2^{+},CD4^{+}, CD8^{+}, CD11b^{+},CD16^{+}, CD19^{+} and CD57^{+} positive cells were followed up to 2W, 4W,12weeks after the operation. The trials were monitored before starting anti-cancer chemotherapy, adjusting the time zone so as to coincide the circadian rhythm of leukocyte. The monitoring tests were set up for CD2^{+}, CD4^{+}, CD8^{+}, CD11b^{+}, CD16^{+}, CD19^{+}and CD57^{+} positive cells were followed up to on and off of CABG, 1d, 3d, 7d, 14 day after the operation. The trials were monitored before starting anti-cancer chemotherapy, adjusting the time zone so as to coincide the circadian rhythm of leukocyte.

Discussion

Hypertension in senile has been associated with cognitive decline and dementia in the late-elderly. In fact, hypertension induces long term structural changes in the deep penetrating small cerebral arteries, and endothelial dysfunction, leading to a silent ischemic process in these vessels, which may be detected as white matter lesions, silent lacunar infarcts and micro-bleeds by magnetic resonance image. The pathophysiology of brain damage induced by high BP is represented. All these lesions evolve for years in a silent manner until the final clinical event in the form of hemorrhagic or ischemic stroke. In addition, there is robust evidence supporting that silent small vessels cerebral disease is also associated with cognitive decline and some immunological factors. The reports showed that this process starts in the middle age in untreated hypertensive and ECC patients. Since then many studies has confirmed the relationship between high BP, structural silent brain lesions and cognitive decline of immunological factors. It may possible to compare the data of infants with congenital heart disease with published data about the neurologic outcome of ECC after premature birth to estimate the predictive value of early ECC analysis on neurodevelopment outcome. Furthermore, we analyze our longtime follow up data of heart rate variability in ECC patient with operated congenital heart disease to proof our approach that focused on the prevention of early life stress due to heart failure in ECC patient with congenital heart disease. We introduce the term autonomic imprinting to understand the lifelong consequences of early life stress on the autonomic nervous system. Age-related deterioration in cardiovascular function is usually attributed to structural and functional changes in the myocardium and associated blood vessels, leading to impairment of left ventricular function, increased after-load and blunted inotropic and chronotropic responses to catecholamine. Therefore, the present study was designed to examine the effect of all-out ECC stress on left ventricular function in ECCG patients. The surgical management of ECC has witnessed a considerable evolution in the past few decreases. The advent of cardio-plastic surgery has brought new dimensions to conserving surgery and included the aesthetic principles of surgery to cardio management [8-14]. The significant developments in the surgical management of cardio-vascular surgery have been paralleled by similar advancements in reconstructive surgery. Earlier when mastectomies where prevailing, it made perfect sense to look for flaps with large volumes of tissue and muscle bulk such in the operative site. The harvest of these flaps often left significant morbidities such as the abdominal wall weakness and the seroma in the back. Nowadays the breast surgeon is more than often faced with smaller defects for which such bulky flaps offer a surplus of tissue with unacceptable morbidities compared to the smaller defects these flaps have to reconstruct. Studies have reported correlations between the recovery from BP and immunological factors [15,16].

Hypertension in senile has been associated with cognitive decline and dementia in the late-elderly. In fact, hypertension induces long term structural changes in the deep penetrating small cerebral arteries, and endothelial dysfunction, leading to a silent ischemic process in these vessels, which may be detected as white matter lesions (WML), silent lacunar infarcts (SLI) and micro-bleeds (MBs) by magnetic resonance image (MRI). The pathophysiology of brain damage induced by high BP is represented in figure 1. All these lesions evolve for years in a silent manner until the final clinical event in the form of hemorrhagic or ischemic stroke. In addition, there is robust evidence supporting that silent small vessels cerebral disease is also associated with cognitive decline and dementia. More than fifteen years ago

we showed that this process starts in the middle age in untreated hypertensive patients [17] [3]. Since then many studies has confirmed the relationship between high BP, structural silent brain lesions and cognitive decline [18]. We now compare the data of infants with congenital heart disease with published data about the neurologic outcome of infants after premature birth to estimate the predictive value of early HRV analysis on neurodevelopment outcome. Furthermore, we analyze our longtime follow up data of heart rate variability in children with operated congenital heart disease to proof our approach that focused on the prevention of early life stress due to heart failure in children with congenital heart disease [19]. We introduce the term autonomic imprinting to understand the lifelong consequences of early life stress on the autonomic nervous system [20]. Age-related deterioration in cardiovascular function is usually attributed to structural and functional changes in the myocardium and associated blood vessels [21], leading to impairment of left ventricular function, increased after-load and blunted inotropic and chronotropic responses to catecholamine [22]. The Wingate Anaerobic Test exposes the subjects to a very high degree of sudden strenuous all-out exercise, and thus, may alter left ventricular function [23]. Therefore, the present study was designed to examine the effect of all-out anaerobic exercise stress on left ventricular function in young and older adult males. The surgical management of breast cancer has witnessed a considerable evolution in the past few decades. Improvements in our knowledge of the vascular anatomy have enabled the design of a new type of cutaneous flaps, which are based on perforating vessels only [24]. Thus, donor site morbidity is markedly reduced. ECC is a type of chronic, systemic autoimmune disease which has the predominant feature of symmetrical polyarthritis, and antiinflammatory analgesic symptomatic treatments, which would delay the development of the disease, offering the major therapeutic options [25]. Local inflammation of the joints is a key link in the process of its pathogenesis; bone loss or damage caused by inflammation is the result of changes to the joints in RA, and symptoms of pain associated with joint swelling are often the most severe symptoms of which the patient complains, although the precise mechanism has yet to be fully elucidated.

Conclusion

AID could be a serious outcome complicating primary ECC is greatly affected by many risk factors. Pre-procedural risk factors and clinical status are the most important and common predictors of developing cardiac impairment post contrast exposure. In this study our patients were evaluated in a mechanical stress resulting typical AIDs. The conclusions were,

1) Total leucocyte was decreased 60 min after CAPG, but recovered 1d, 3d, 7d and 14 days after ECC (P<0.05).
2) Total lymphocyte number were not different around CAPG. However, down regulated 1d, 3d, 7d and 14days (P<0.05).
3) CD positive cell was down regulated 1d, 3d, 7d and 14 days after operation.
4) All the CD positive cell was significantly affected by around CAPG operation.
    1. There was no significant difference in immunological factors between NH and HG group.

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