PB-02

Analysis of HLA allele in infertile patients from Taiwan population

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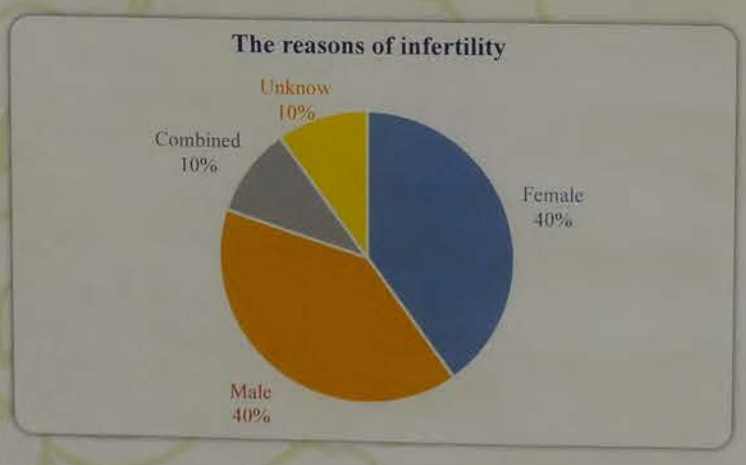
Introduction

The reasons of infertility are very complex. Sperm immobilizing antibody (SIA) in the sera of women cause low pregnancy rate. HLA alleles DR and DQ may account for higher frequency in SIA groups. Our purpose was to investigate the distributions of HLA alleles and estimate their associations with infertile women in Taiwan population. We analyzed HLA database of 100 infertile couples from Chi-Mei Hospital in southern Taiwan. HLA typing was performed by polymerase chain reaction sequence specific primer (PCR-SSP) method. The mean age of female infertile patients was 37.8±7.5 years. There were 19 genes relative frequency for HLA-DRB1 and DQB1 in contrast to normal population. The frequency of HLA DRB1*0301 (DR17) was 26.0% in infertile women, as compared to the reference normal group (14.5%). The patients group was higher ratio than normal group in 1.8X. A HLA-DQB1*0201 (DQ02) gene frequency of 26.0% was found in the infertile women in contrast to 19.0% in the normal population. These results showed an increase in HLA DR17 and DQ02 alleles in the women patients compared with the control population. On the other hand, we analyzed the HLA alleles sharing of 100 couples. There was a highly significant similar of HLA sharing in the couple who have 7 HLA sharing antigens. There were 10 couples shared there HLA antigens, 15 couples shared four HLA antigens, and 5 couples shared five HLA antigens. The some cases of infertility might be caused by closed histocompatibility between partners, it can be performed desensitization therapy effectively. The high frequency of HLA-DRB1*0301 DQB1*0201 among the patients may account for a higher frequency of SIA in the southern Taiwan population. Since the causes of infertility are many, HLA typing used can help clear reason of infertile couples.



Materials and methods

Patients and HLA typing

One hundred infertile couples from Chi-Mei Hospital in Taiwan population were selected. We analyzed HLA database of 100 infertile couples. The mean age of female infertile patients was 37.8±7.5 years. DNA sample of patients were obtained from peripheral white blood cells. HLA typing was performed by polymerase chain reaction sequence specific primer (PCR-SSP) method. The PCR were accomplished on Applied Biosystems 2720 Thermal Cycler system and using by Invitrogen AllSet+ Gold SSP HLA-ABC and DRDQ Low Res Kit.

Results

There were 19 genes relative frequency for HLA-DRB1 and DQB1 in contrast to normal population. The frequency of HLA DRB1*0301 (DR17) was 26.0% in infertile women, as compared to the reference normal group (14.5%). The patients group was higher ratio than normal group in 1.8X. No gene frequency differences were observed in any other HLA DRB1 genes between the patients and reference normal group. (Table1)

Table 1. The compare with HLA DRB1 frequency and reference normal group

	DR1	DR4	DR7	DR8	DR9	DR11	DR12	DR13	DR14	DR15	DR16	DR17
n (n=100)	3	31	3	21	26	12	28	9	12	19	10	26
HLA frequency	3.0%	31.0%	3.0%	21.0%	26.0%	12.0%	28.0%	9.0%	12.0%	19.0%	10.0%	26.0%
Reference normal group	1.0%	32.7%	5.5%	21.6%	33.3%	12.7%	25.5%	12.7%	18.2%	23.6%	14.7%	14.5%

A HLA-DQB1*0201 (DQ02) gene frequency of 26.0% was found in the infertile women in contrast to 19.0% in the normal population.(Table2) These results showed an increase in HLA DR17 and DQ02 alleles in the women patients compared with the control population.

Table 2. The compare with HLA DQB1 frequency and reference normal group

	DQ2	DQ4	DQ5	DQ6	DQ7	DQ8	DQ9
n (n=100)	26	17	26	41	41	19	30
HLA frequency	26.0%	17.0%	26.0%	41.0%	41.0%	19.0%	30.0%
Reference normal group	19.0%	19.0%	30.0%	33.0%	46.0%	20.0%	31.0%

There was a highly significant similar of HLA sharing in the couple who have 7 HLA sharing antigens. There were 10 couples shared there HLA antigens, 15 couples shared four HLA antigens, and 5 couples shared five HLA antigens. (Table3) The some cases of infertility might be caused by closed histocompatibility between partners, it can be performed desensitization therapy effectively.

Table 3. HLA sharing in the couples studied

Sharing of HLA		
Antigen	n	%
3	10	10.0%
4	15	15.0%
5	5	5.0%
7	1	1.0%

As difference populations have different HLA polymorphism, investigation of the relationship of other HLA genes and idiopathic male infertility with large sample size, is warranted in the future.

Discussion

Infertility is a complex disease resulting from multiple interactions between gene and other unknown reasons. In the world, there were more and difference populations that have different HLA polymorphism. In the southern Taiwan, the high frequency of HLA-DRB1*0301 and DQB1*0201 among the patients may account for a higher frequency of SIA. Since the causes of infertility are many, HLA typing used can help clear reason of infertile couples.

Reference

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2. Yoshiyuki Tsuji, Mizumi Mitsuo, Reiko Yasunami, et al. HLA DR and DQ gene typing of infertile women possessing sperm immobilizing antibody. Journal of Reproductive Immunology (2000) 46:31-38
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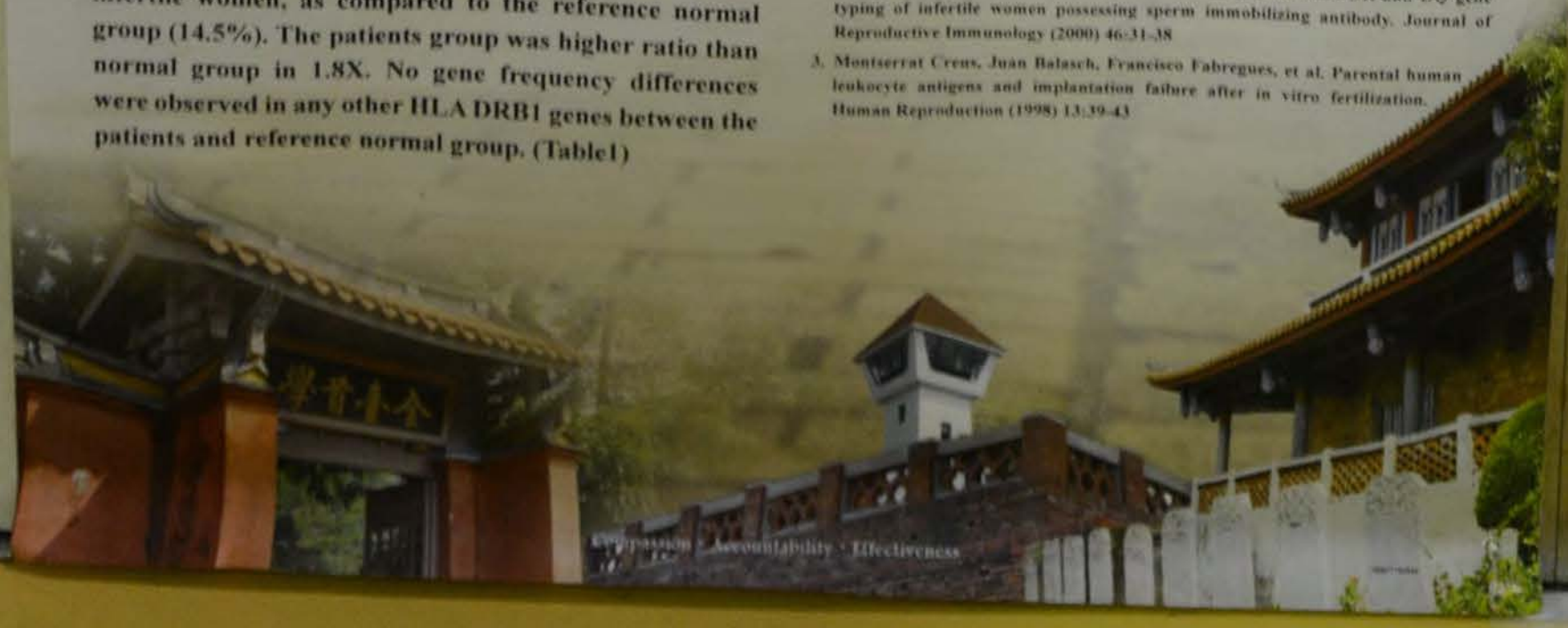

Immunology

PB-03

HCV Genotype Change Conferred Treatment Failure : A Case Report

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1. INTRODUCTION

Hepatitis C Virus (HCV) is an enveloped RNA virus; its high genetic diversity often brings about difficulties in drug therapy and prognosis. In this study, we describe a case report of HCV genotype change at virologic failure.

2. METHOD & RESULT

A 73-year-old man, with prior chronic hepatitis, visited our hospital on Jan. 23, 2014. abdominal sonography and Computerized Tomography demonstrated hepatocellular carcinoma, hence transcatheter arterial embolization was performed for therapy on Feb. 27. Follow-up laboratory tests revealed rising AST and ALT level: 45,61 IU/mL on Mar.12; 54,92 IU/mL on Jun.25 respectively; with HCV viral load up to 1.36 x10^5 IU/mL (on Jun.25). On Jul.18 and Aug.4, blood specimens were successively refered to an institution, qualified by TAF, for HCV RNA genotype analysis by real-time polymerase chain reaction (PCR) methods. Twice tests with double check proved to be mixed genotypes of 1 (non-subtype 1a,1b) and 4. Combination of pegylated interferon and ribavirin started since Aug.11. 4-week therapy appeared to be effective initially, as sustained blood monitoring displayed declining levels in AST,ALT and HCV viral load(24 IU/mL,21 IU/mL,420 IU/mL respectively). AST and ALT levels rose again subsequently. HCV viral load levels at week 11,21 also showed an upward trend (3.83 x10^4,4.49x 10^5 IU/mL). RNA genotype 1a upon retest on Jan. 5, 2015 were thus confirmed both by PCR as well as sequencing technology. Eventually medications were stopped due to treatment failure at week 40. Follow-up tests on Mar. 23, 2016 still remained genotype 1a, with persistently rising HCV viral load up to 5.53 x10^5 IU/mL.

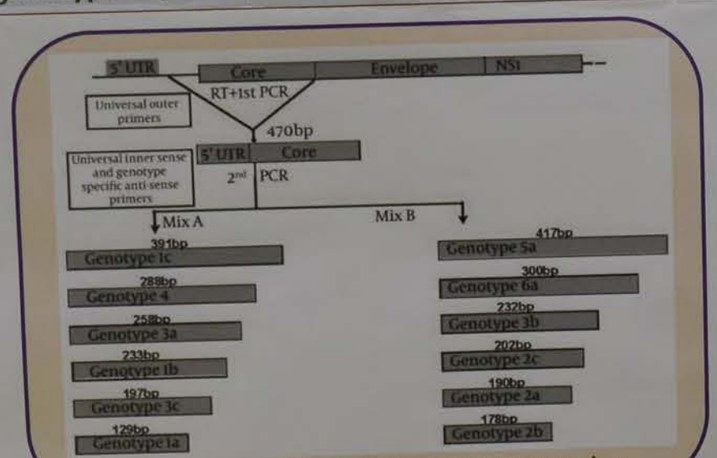

Fig1. Type-specific PCR for HCV Genotyping

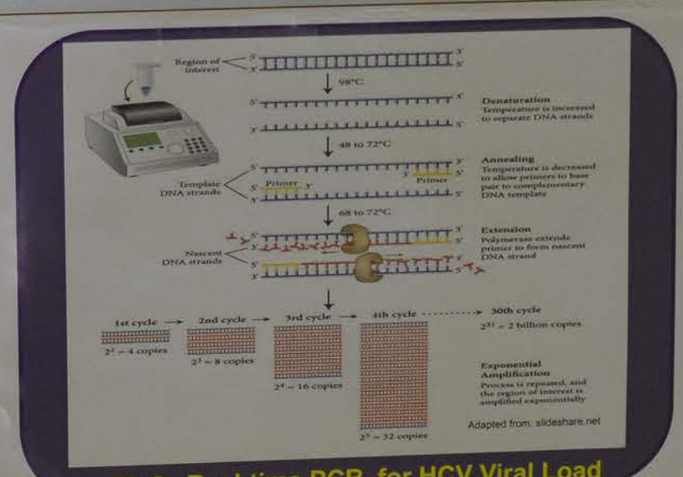

Fig2. Real-time PCR for HCV Viral Load

Date in 2014	3/12	6/25	7/18	8/4
AST	45	54		
ALT	61	92		
HCV Viral Load		1.36x10^5		
HCV Genotype			Mixed 1+4	

Treatment Started on 8/11

Therapy week	Week 4	Week 11	Week 11	Week 40
AST	24	36	59	Treatment Stopped, Remain Type 1a
ALT	21	43	88	
HCV Viral Load	420	3.84x10^4	4.49x10^5	
HCV Genotype			1a	

Fig3. Laboratory Data Development in Clinical Course

3. CONCLUSION

Genotype change, owing to adaptive mutations under drug pressure, might contribute to treatment failure. Accumulated drug resistance information could aid in management guidance or vaccine development.

Immunology

PB-04

Immunology

PB-06

A case of prozone effect on PRA

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Background

- Donor-specific HLA antibody(DSA) can cause antibody-mediated rejection (ABMR).
- DSA is also a powerful markers to predict ABMR after transplantation.
- Many laboratories use Luminex PRA to detect DSA.
- Luminex PRA is a highly sensitive method, but it also has some limitations.
- Here, we report a case with prozone effect on PRA.

Case

- A 45-year old man had kidney transplantation at 2009.
- October 2014
  - His creatinine increased to 1.49 mg/dl
  - PRA: DQ7 DSA (MFI 5,944)
  - Kidney biopsy: ABMR
- November 2014
  - Treated with Rituximab, intravenous immunoglobulin and four sessions of plasma exchange.
- December 2014
  - PRA: DQ7 DSA (MFI 19,807)

- Because his DSA MFI increased significantly after desensitization, we suspected PRA result at October 2014 may be due to prozone effect.

- Ten-fold dilution of the sample drawn at October were done to confirm the prozone effect, and DQ7 DSA (MFI 18,084) was identified.

Conclusion

- HLA molecules on cell surface are floating in the cell membrane.
- This motility facilitates the binding of antibodies.
- However, HLA molecules on beads are bound rigidly to the surface and cannot move.
- Therefore, the density of HLA antigens on beads can influence the binding of antibodies.

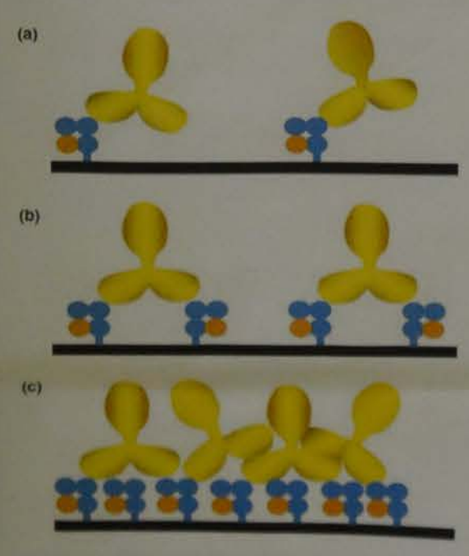

Figure 1. The impact of the antigen density on antibody binding in Luminex PRA: (a) Low antigen density. (b) High antigen density may increase the sensitivity of an immunoassay. (c) Very high antigen density may lead to tightly packed antibodies which might interfere with binding of the detection antibodies and increase the chance of prozone. Reproduced from *Int J Immunogenet*. 2013 Jun;40(3)

- The high HLA density on beads increases sensitivity, but it also increases risk of prozone effect.
- If the antibody titer is very high in patient serum, and HLA density on beads are high, antibodies can be packed around the beads, resulting univalent binding.
- Because univalent binding is not stable, it could be washed away through washing steps, resulting unexpected low MFI.
- Most patients who receive desensitization have high titer of HLA antibodies.
- Therefore, it is recommended to test sample with 10-fold dilution together in monitoring desensitization effect.

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Immunology

PB-08

PB-08

Studies on immune responses of macrophage to the extracellular polysaccharide of *Vibrio vulnificus*

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Backgrounds

1. Macrophages are component of innate immunity, and with multiple functions in both inhibiting or promoting cell proliferation and tissue repair.
2. Macrophages can develop distinct functional phenotypes via undergoing different phenotypic polarization, including classically activated macrophages (M1) and alternatively activated macrophages (M2).
3. M1 macrophages are characterized by the secretion of pro-inflammatory cytokines, including IL-1β, and TNF-α.
4. M2 macrophages induce a weak immune response and with the production of interleukin-10.

The induction of inflammation-related cytokines among of *Vibrio vulnificus*

Vibrio vulnificus

1. Wild-type strain (YJ016),
2. Capsular polysaccharide (CPS) mutant strain (JF045)
3. Complementary strain (JF049)

Results

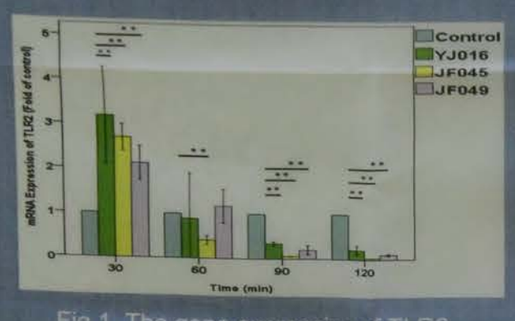

Fig 1. The gene expression of TLR2 to RAW 264.7 cell induced by *Vibrio vulnificus*

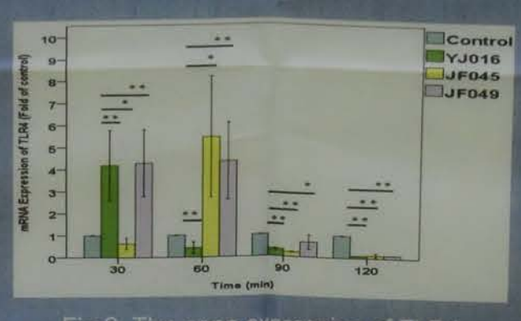

Fig 2. The gene expression of TLR4 to RAW 264.7 cell induced by *Vibrio vulnificus*

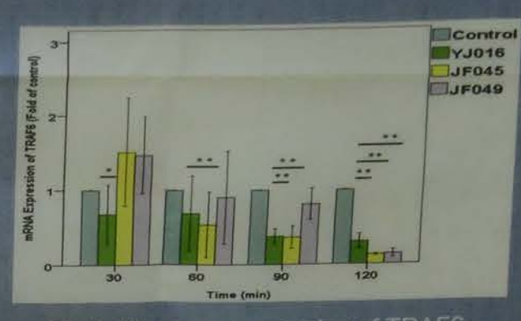

Fig 3. The gene expression of TRAF6 to RAW 264.7 cell induced by *Vibrio vulnificus*

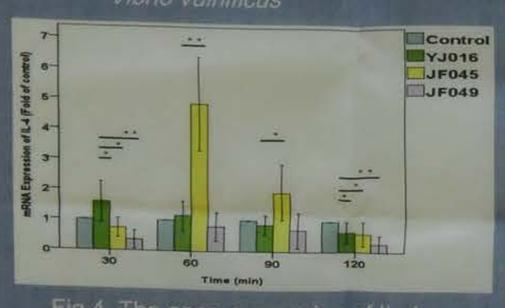

Fig 4. The gene expression of IL-4 to RAW 264.7 cell induced by *Vibrio vulnificus*

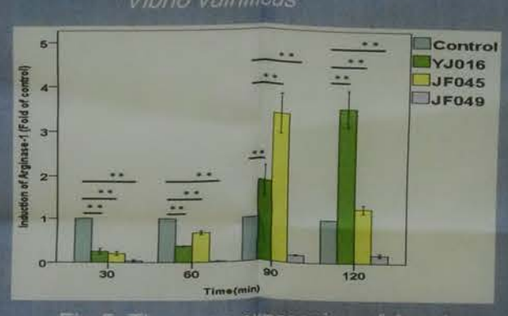

Fig 5. The gene expression of Arg-1 to RAW 264.7 cell induced by *Vibrio vulnificus*

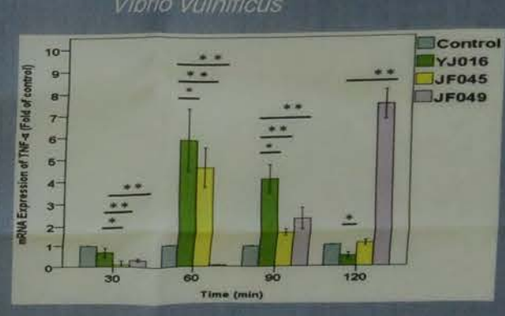

Fig 6. The gene expression of TNF-α to RAW 264.7 cell induced by *Vibrio vulnificus*

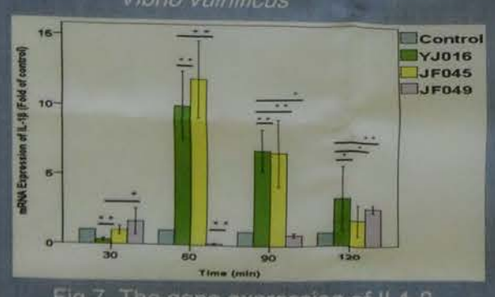

Fig 7. The gene expression of IL1-β to RAW 264.7 cell induced by *Vibrio vulnificus*

Conclusions

1. The *Vibrio vulnificus* wild-type strain (YJ016) induced RAW 264.7 cells with upregulation of signals in a time dependent manner.
2. The signal regulations of the complementary strain (JF049) were later than those of wild-type strain (YJ016).
3. The results help us to understand the effect of EPS on the M1-M2 polarization of macrophages and the time dependent manner.

Immunology

PB-09

Immunology

PB-10

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PB-10

Comparison of Indirect Immunofluorescence Assay and EliA CTD Screen for Measurement of Antinuclear Antibodies

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Introduction

Indirect immunofluorescence (IFA) on HEp-2 cells is the reference method for screening antinuclear antibodies (ANA) in clinical laboratory routine. However the method is time-consuming, and requires experienced analysts. EliA CTD screen (ECS, Thermo Fisher) is the fluorescence enzyme immunoassay (FEIA) for detection of ANA against human antinuclear antigens (U1RNP, SS-A/Ro, SS-B/La, centromere B, Scl-70, Jo-1, Fibrillarin, RNA Pol III, Rib-P, PM-Scl, PCNA, Mi-2, Sm, and dsDNA), and it's suitable for the high-throughput screening of ANA. Therefore, the present study aimed to evaluate the agreement of IFA and ECS in ANA screening, and to determine whether ECS can replace IFA.

Materials & Methods

- From 2014 to 2015, 711 serum samples were collected from the patients with suspected connective tissue disease (CTD) in Taipei Medical University-Shuang Ho Hospital.
- All samples were then examined simultaneously using IFA (FLUORO HEPANA TEST, MBL) and ECS.
- According to the results of ANA screening tests, some patients were further examined by Phadia EliA assays for quantitative detection of ANA, containing dsDNA, SSA/Ro, SSB/La, Sm, and RNP.

Results

- Of 711 samples, 73 (10.3%) were positive by IFA, and 78 (11.0%) were positive by ECS.
- After exclusion of all ECS-equivocal results, ECS had sensitivity of 58.6%, specificity of 94.0%, positive predictive value of 52.6%, negative predictive value of 95.3%, accuracy of 87.8%, positive likelihood ratio of 9.81, negative likelihood ratio of 0.44, and kappa coefficient of 0.434, when IFA was used as the reference method for ANA screening (Table 1).
- Among the cases of common IFA patterns, 100% centromere pattern (9/9), 73.3% speckled pattern (11/15), 25% homogeneous pattern (2/8), and 22.2% nucleolar pattern (2/9) were observed with the ECS positive results (Table 2).
- As shown in Figure 2, patients with anti-SSB/La (n=7), anti-Sm (n=3), or anti-RNP (n=4) were positive for both IFA and ECS. There were 94.1% cases with ECS positive results (n=34) and 47.0% cases with IFA positive results (n=16) in 34 patients with anti-SSA/Ro. Of 14 patients with positive anti-dsDNA assay, 92.9% cases were positive for ECS (n=13) and 57.1% cases were positive for IFA (n=8).

Table 1. Agreement between IFA and ECS in the suspected CTD patients.

EliA CTD screen[a]	Indirect immunofluorescence on HEp-2 cells[b] Negative	Positive	Total
Negative	583	29	612
Equivocal	18	3	21
Positive	37	41	78
Total	638	73	711

[a] The results of ECS were reported as negative (ratio <0.7), equivocal (ratio 0.7-1.0), or positive (ratio >1.0).
[b] The IFA titer at a dilution equal to or greater than 1:160 was regarded as positive.

Table 2. Results of IFA patterns and ECS in the IFA-positive patients.

IFA patterns	EliA CTD screen Negative	Equivocal	Positive	Total	Positive ratio (%)
Centromere	0	0	9	9	100.0
Mixed homogeneous + centromere + cytoplasmic	0	0	1	1	100.0
Mixed homogeneous + cytoplasmic	0	0	2	2	100.0
Mixed homogeneous + nucleolar + cytoplasmic	0	0	1	1	100.0
Mixed nuclear dot + cytoplasmic	0	0	1	1	100.0
Mixed speckled + cytoplasmic	0	0	2	2	100.0
Mixed speckled + nucleolar	0	0	3	3	100.0
Mixed centromere + cytoplasmic	0	1	3	4	75.0
Speckled	4	0	11	15	73.3
Mixed speckled + homogeneous + cytoplasmic	2	0	1	3	33.3
Mixed speckled + homogeneous	5	1	2	8	25.0
Homogeneous	5	1	2	8	25.0
Nuclear dot	3	0	1	4	25.0
Nucleolar	7	0	2	9	22.2
Mixed homogeneous + nucleolar	2	0	0	2	0.0
Mixed nucleolar + cytoplasmic	1	0	0	1	0.0
Total	29	3	41	73	56.2

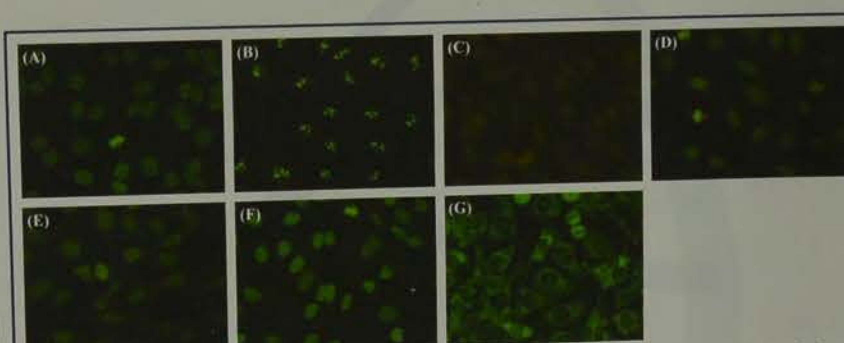

Figure 1. The cases of IFA-positive patterns were frequently observed with ECS-negative results. (A) Homogeneous pattern. (B) Nucleolar pattern. (C) Nuclear dot pattern. (D) Mixed speckled and homogeneous pattern. (E) Mixed speckled, homogeneous and cytoplasmic pattern. (F) Mixed homogeneous and nucleolar pattern. (G) Mixed nucleolar and cytoplasmic pattern.

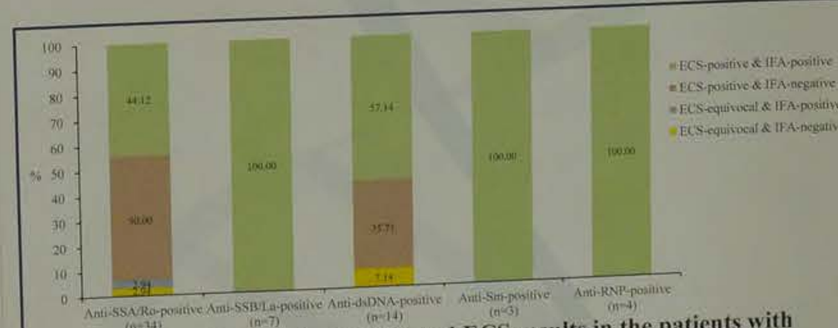

Figure 2. Discordance between IFA and ECS results in the patients with anti-SSA/Ro, anti-SSB/La, anti-dsDNA, anti-Sm, or anti-RNP antibodies.

Conclusion

- ECS exhibited excellent specificity and negative predictive value for detection of ANA from serum.
- The ECS results were well consistent with the IFA results that were positive with centromere pattern (common in CREST) or speckled pattern (common in SLE, MCTD, and SjS), but the results were inconsistent with the IFA results that were positive with homogeneous pattern (common in SLE, drug-induced lupus, RA, and Sjs) or nucleolar pattern (common in SSc, SLE, and SjS).
- The inconsistent results may be due to that antigen panel of ECS not includes histone and nucleosome antigen.
- The IFA and ECS results for patients with anti-SSB/La, anti-Sm, or anti-RNP exhibited good agreement, and ECS had a better ability to detect anti-SSA/Ro and anti-dsDNA than IFA.
- In conclusion, IFA and ECS complement each other, so a combination of both assays can provide better performance of ANA testing.

Immunology

PB-11

Combining serum Procalcitonin with Lactic Acid as biomarkers to elevate the diagnosis of bacteremia

Introduction

Bacteremia is the presence of bacteria in the blood which is always abnormal. The immune response to the bacteria can cause sepsis and septic shock, which has a relatively high mortality rate. Therefore, it's a high priority for the doctors to diagnose bacteremia. Bacteremia is most commonly diagnosed by blood culture. However, blood culture usually take 2-5 days for bacteria to multiply in order to be detected. We want to find out whether there are other biomarkers that can help diagnose bacteremia and can be detected in blood sample in just a few hours after blood collection. Measurement of procalcitonin can be used as a marker for infection caused by bacteria although levels of procalcitonin in the blood are very low. In our study, we want to use another biomarker to increase the diagnosis of bacteremia, ex: the level of lactic acid. Lactic acid gets higher when strenuous exercise or severe infection.

Figure 2: Pie chart of bacteria distribution from bacteremia patients' blood culture results.

	GNB	GPC	GPB	Total case
PCT mean	23.12	14.2	6.6	14.64
Case number of PCT >0.5ng/ml	84	60	8	152
Percentage of PCT >0.5ng/ml	88.42%	75%	80%	82.16%
Case number of PCT> 10ng/ml	30	12	2	44
Case number of Blood culture(+)	95	80	10	185

Table 1: The mean, case number and percentage of PCT over 0.5 ng/ml and number of PCT over 10 ng/ml in different bacteria gram stain groups.

Method

We collected 185 bacteremia patients' samples (including inpatients and outpatients) from 2015/01/01 to 2015/12/31 in our hospital. Then we tested patients' procalcitonin, lactic acid level and also ran blood culture.

Results

First, we analyzed the bacteria distribution of our hospital patients' blood culture sample (figure 1 and 2). We found out that GNB account for the most proportion, up to 51%. Next we want to observe whether the PCT in all the sample have reach the cut off value, which is above 0.5 ng/ml (table 1). As we can see, the average percentage were about 80%. And we also calculate the mean of PCT in different bacteria group (table 1 and figure 4a). What's more, we want to exam the case number in which have a higher PCT level (over 10 ng/ml; table 1). Then, we want to add a supplement biomarker-lactic acid (cut off value is 2.2 mmol/L), to see whether the sensitivity will elevate (figure 3). Last, we calculate the mean of lactic acid in three different bacteria group (figure 4b).

Figure 3: Bar graph of sensitivity by comparing procalcitonin as a single biomarker verse combination of lactic acid as a supplement biomarker.

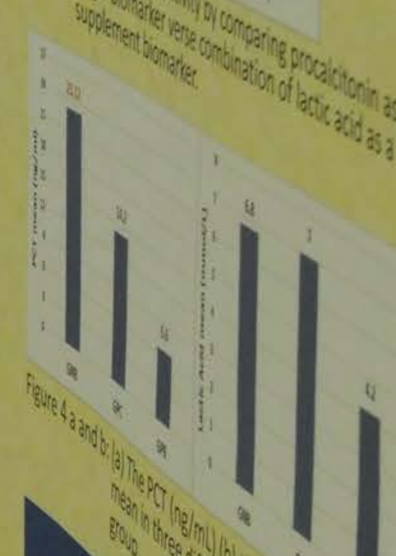

Figure 4 a and b: (a) The PCT (ng/mL) (b) the lactic acid (mmol/L) mean in three different bacteria gram stain group.

Figures and Table

Summary

All blood culture came out positive with bacteria. The procalcitonin level range from 0.0602 to 266.8 ng/mL. By using procalcitonin as a biomarker (cut off: 0.5 ng/mL), the sensitivity was about 80%. If we combine lactic acid as a supplement biomarker (cut off: 2.2 mmol/L), the sensitivity would elevate to 92%. In addition, out of the 44 patients who had a higher procalcitonin level (over 10 ng/mL), 30 patients' blood culture result in GNB growth. What's more, we can observe that the average of PCT mean in GNB are much higher than both GPC an GPB. However, in the mean of lactic acid result, no such pattern are seen. In the future, we want to test the level of procalcitonin and lactic acid in blood culture results negative bacteremia patients to ensure our finding.

Immunology

PB-11

Combining serum Procalcitonin with Lactic Acid as biomarkers to elevate the diagnosis of bacteremia

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Introduction

Bacteremia is the presence of bacteria in the blood which is always abnormal. The immune response to the bacteria can cause sepsis and septic shock, which has a relatively high mortality rate. Therefore, it's a high priority for the doctors to diagnose bacteremia. Bacteremia is most commonly diagnosed by blood culture. However, blood culture usually take 2-5 days for bacteria to multiply in order to be detected. We want to find out whether there are other biomarkers that can help diagnose bacteremia and can be detected in blood sample in just a few hours after blood collection. Measurement of procalcitonin can be used as a marker for infection caused by bacteria although levels of procalcitonin in the blood are very low. In our study, we want to use another biomarker to increase the diagnosis of bacteremia, ex: the level of lactic acid. Lactic acid gets higher when strenuous exercise or severe infection.

Method

We collected 185 bacteremia patients' samples (including inpatients and outpatients) from 2015/01/01 to 2015/12/31 in our hospital. Then we tested patients' procalcitonin, lactic acid level and also ran blood culture.

Results

First, we analyzed the bacteria distribution of our hospital patients' blood culture sample (figure 1 and 2). We found out that GNB account for the most proportion, up to 51%. Next we want to observe whether the PCT in all the sample have reach the cut out value, which is above 0.5 ng/ml (table 1). As we can see, the average percentage were about 80%. And we also calculate the mean of PCT in different bacteria group (table 1 and figure 4a). What's more, we want to exam the case number in which have a higher PCT level (over 10 ng/ml; table 1). Then, we want to add a supplement biomarker- lactic acid (cut off value is 2.2 mmol/L), to see whether the sensitivity will elevate (figure 3). Last, we calculate the mean of lactic acid in three different bacteria group (figure 4b).

Figures and Table

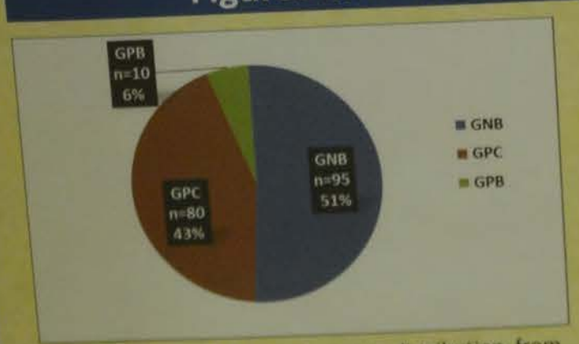

Figure 1: Pie chart of bacteria group distribution from bacteremia patients' blood culture results.

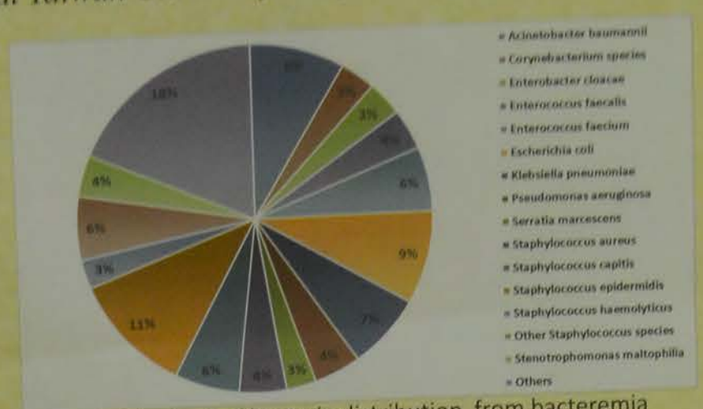

Figure 2: Pie chart of bacteria distribution from bacteremia patients' blood culture results.

	GNB	GPC	GPB	Total case
PCT mean	23.12	14.2	6.6	14.64
Case number of PCT >0.5ng/ml	84	60	8	152
Percentage of PCT >0.5ng/ml	88.42%	75%	80%	82.16%
Case number of PCT> 10ng/ml	30	12	2	44
Case number of Blood culture(+)	95	80	10	185

Table 1: The mean, case number and percentage of PCT over 0.5 ng/mL and number of PCT over 10 ng/ml in different bacteria gram stain groups.

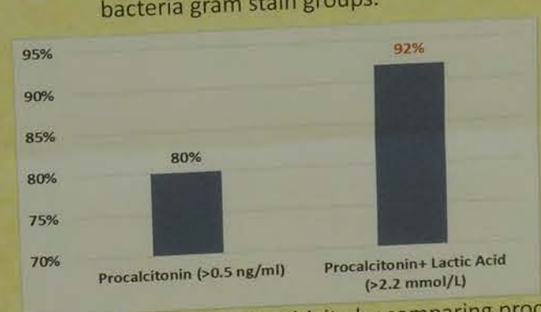

Figure 3: Bar graph of sensitivity by comparing procalcitonin as a single biomarker verse combination of lactic acid as a supplement biomarker.

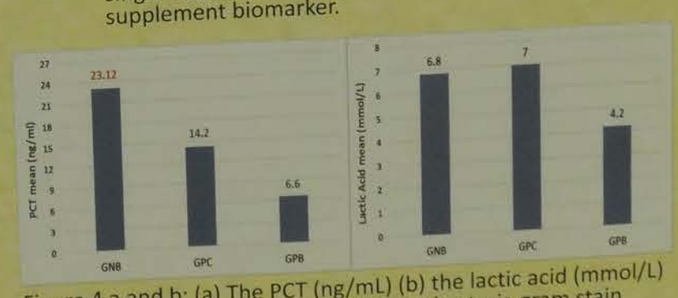

Figure 4 a and b: (a) The PCT (ng/mL) (b) the lactic acid (mmol/L) mean in three different bacteria gram stain group.

Summary

All blood culture came out positive with bacteria. The procalcitonin level range from 0.0602 to 266.8 ng/mL. By using procalcitonin as a biomarker (cut off: 0.5 ng/mL), the sensitivity was about 80%. If we combine lactic acid as a supplement biomarker (cut off: 2.2 mmol/L), the sensitivity would elevate to 92%. In addition, out of the 44 patients who had a higher procalcitonin level (over 10 ng/mL), 30 patients' blood culture result in GNB growth. What's more, we can observe that the average of PCT mean in GNB are much higher than both GPC an GPB. However, in the mean of lactic acid result, no such pattern are seen. In the future, we want to test the level of procalcitonin and lactic acid in blood culture results negative bacteremia patients to ensure our finding.

Immunology

PB-12

Anorexia in the elderly and its association with latent inflammation.
The roles of inflammatory cytokines and appetite-related hormones in the pathogenesis of anorexia of aging.

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OBJECTIVES

A certain proportion of the elderly people suffer from anorexia without evident etiology. To understand the pathogenesis of anorexia in these elderly people, we investigated the relation of appetite status to serum levels of appetite-related hormones and inflammatory cytokines. We also investigated the influence of hunger hormone, ghrelin, on the inflammatory responses in the mouse liver injury models.

METHODS

① Twenty-four elderly people with or without anorexia (>65 yrs) were enrolled.

Group

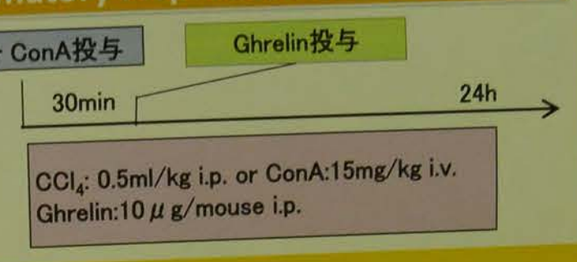
	Controls (without anorexia)	Patients (with anorexia)
Average age	72.6±4.4	77.9±10.1
male/female	7 / 10	3 / 4

Analaysis
- the assessment test of nutrition (MNA)
- body measuring
- blood tests
  hematology, biochemistry, hormones, cytokines

② Investigation for regulatory function of ghrelin on inflammatory responses in mouse model.

We conducted mouse experiments in which we tested the effect of ghrelin administration on CCl_4- and concanavalin A (ConA)-induced liver injury

C57BL/6 8-10week ♂
5-7 mice/group

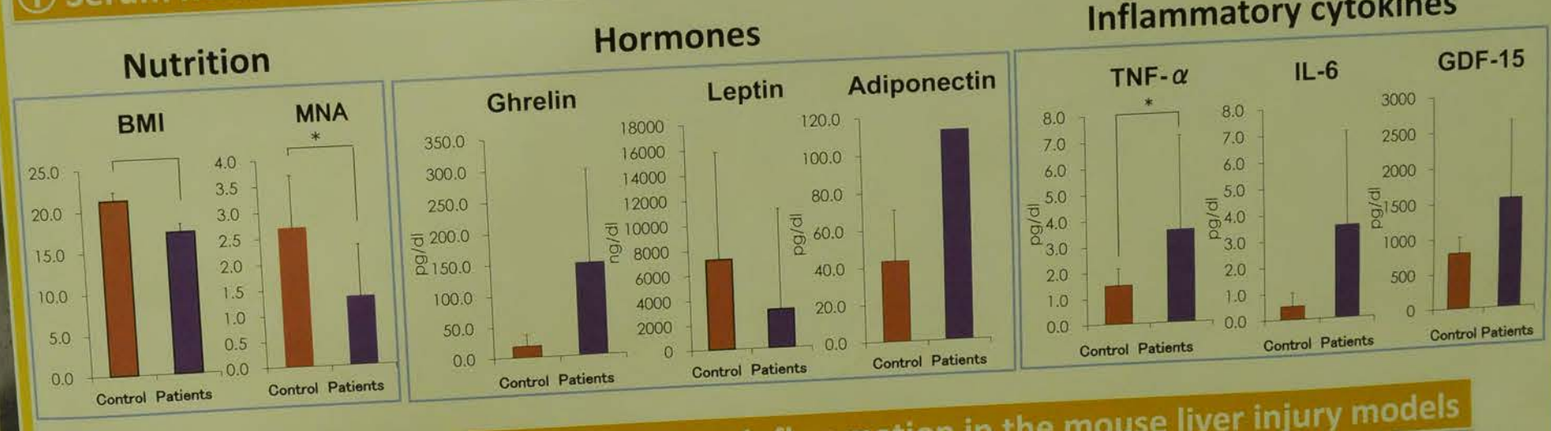


RESULTS

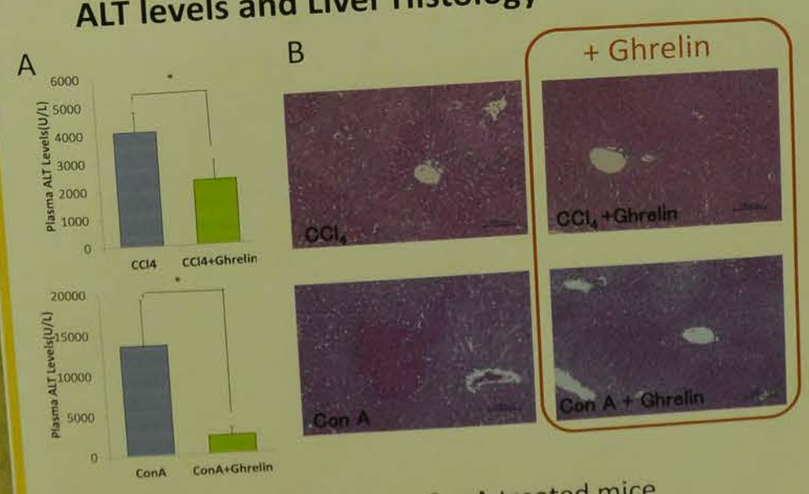
① Serum hormone and cytokine levels in the patients with anorexia.

Correlations between Inflammatory cytokines

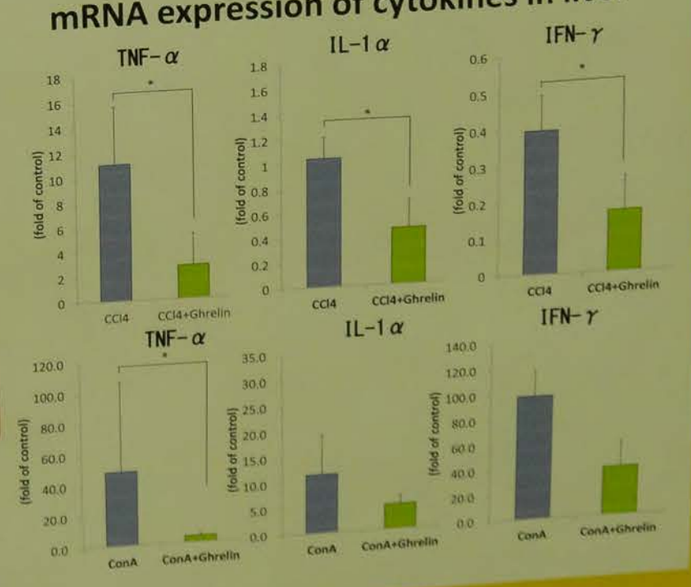
	TNF-α	IL-6	GDF-15	CRP
TNF-α				
IL-6	0.463**			
GDF-15	0.419**	0.326**		
CRP	0.097	0.446**	0.409**	



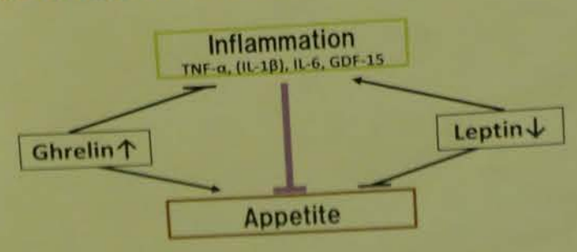
② Ghrelin administration ameliorated liver inflammation in the mouse liver injury models



A: Serum ALT levels of CCl_4 or ConA treated mice
B: Liver Histology of the mice CCl_4 or ConA hepatitis model



HYPOTHETICAL MECHANISM



CONCLUSIONS

The elevation of serum TNF-α levels in the patients suggested that anorexia in the elderly was an inflammation-based clinical condition. As ghrelin was proved to exert immune-regulatory action in the mouse models, tendency of ghrelin elevation in the patients might be a compensatory reaction against the latent inflammation.

PB-13

Comparison of Indirect Immunofluorescence Assa
CTD Screen for Measurement of Antinuclear An

Introduction

Materials & Methods

Results

Conclusion

Figure 1. The cases of IFA-positive patterns were freq
ECS-negative results.

Figure 2. Discordance between IFA and ECS results in
anti-SSA/Ro, anti-SSB/La, anti-dsDNA, anti-Sm, or an

Table 2. Agreement between IFA and ECS in the suspected CTD

Immunology

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PB-14

Presence of high-levels of $CD8^{dimCD3-CD4-}$(NK8) cells is associated with undetectable viral load in HIV infected patients post-HAART

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*Both authors had the same contribution for the paper.

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Introduction

In post-highly active antiretroviral therapy (HAART) therapy the recuperation in human immunodeficiency virus (HIV) patients depends on several factors, were the immune system has a key role, contributing for infectivity and capacity spread reduction. The CD3/CD4/CD8 cellular populations in HIV patients in some cases, show an increase of $CD8^{dim}/CD3^{-}/CD4^{-}$ (NK8) cells above normal values.

Aims

Relate NK8 cells number in HIV patients regarding the evolution of infection, taking in consideration the trinomial: viral load, TCD4 number post-HAART and cell phenotype.

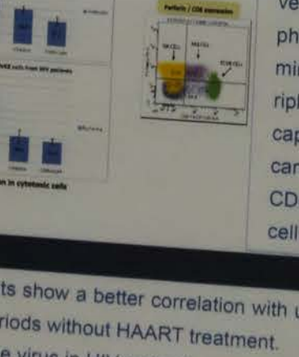
Material and Methods

Study Population

- 518 HIV+ patients post-HAART
- 30 HIV+ patients NK8 cells >8%
- 15 healthy individuals as controls

Techniques

- Association values
- Direct immunofluorescence
- Cytometric analysis
- Cellular activation



Determinations

- - Viral load
  - CD4/CD8 cells
  - Periodes post HAART
- - *Panel 1: CD3/CD8/CD57/NKp46/CD69/CD56/CD16*
  - *Panel 2: CD3APC/CD8/CD45RO/CD45RA/TCRαδ/TCRγδ*
  - *Panel 3: CD3/CD8/CD57/Perforina/CD56 CD16/CD62L*
  - *Panel 4: CD3/CD8/Perforina/CD56+CD16/CD69/ INFγ*
- INFγ

Results&Discussion

Fig.1: NK8 cells population distribution <2% / 2-8%/>8% of cells
Fig.2: NK8 cells correlation of the level of viral load
Fig.3: NK8 cells related to the level of viral load gender.
Fig.4: NK8 cells related to ratio CD4/CD8
Fig.5: NK8 cells related to CD4 or CD8 cells
Fig.6: Periods of negative viral load in the values of
Fig.7: NK8 cells phenotyping
Fig.8: Perforin expression in cytotoxic cells

Discussion

In this study we demonstrated that in the presence of NK8 cells the CD4/CD8 quotient improves, the viral load decreases and compensates the levels of the CD4 lymphocytes *versus* the CD8 ones, decreasing their proportion. As the periods with viral load <37 are significantly longer with NK8 cells and considering the long-term HAART therapy is less effective in a significant proportion of HIV patients, a possible way of supplementary treatment with HAART it would be the immunotherapy with NK8 cells. The NK8 cells have a high expression of CD45RA emphasizing their naive status, only a small percentage progress CD45RA/RO (memory) during the contact and action process against target cells. It may have a regulatory role and it may be IFN-γ producers regarding the marker CD57, 72,2 ± 31% of the NK8 cells are going to express it. There is a relation between high levels of cytotoxicity with the expression of CD69 on cytotoxic cells. In our study, not all the NK8 cells express similar levels of CD69 (47,9±5%) not keeping a direct relation with the levels of cytotoxicity (perforin) found in the study (86,5±4%). A similar phenomenon is observed with the CD62L marker that would determine the action capacity at the central level (lymphoid tissue) or peripheral (target tissue) of certain cytotoxic cells and their migratory capacity, particularly in the $CD56^{dim}$. Therefore, it is evident that we can propound that within the NK8 cells group are subpopulations CD62L/CD56dim with mixed function of interferon γ production, cellular proliferation and cytotoxic action during the viral infection.

Conclusion

⇒ Values above 2 to 8% of NK8 cells in HIV patients show a better correlation with undetectable viral load, favouring the CD4/CD8 quotient. Allowing the immune system regeneration for longer periods without HAART treatment.
Suggesting better prognosis in non-progression of the virus in HIV patient's post-HAAT. The assessment NK8 cells should be considered as another biomarker in HIV infection.

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Immunology

PB-16

Procalcitonin as a biomarker in urine tract infection diagnosis

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Introduction

Urinary tract infection(UTI) is one of the most common infectious disease in all ages. When patients with suspected UTI, doctors will often prescribe urine routine examination and urine culture to assist in the diagnosis. However, many external factors affecting the urinalysis report (for example: specimen collection), and also urine culture needs more time to identify.

Procalcitonin(PCT) is composed of 116 amino acids, is mainly secreted by the thyroid C-cell. Under normal metabolism, PCT need to break down through proteolysis into calcitonin before to release into the blood circulation, and therefore PCT content in the normal blood circulation is very small. However, after 2004 there is a lot of literature research shows that when people affected by a bacterial infection, PCT concentration in the blood significant increased in 2-3 hours. PCT may be produced by immune cells and endothelial cells. When inflammation of the body are stimulated, particularly bacterial infection or sepsis, PCT in the blood can be detected. PCT test can have a preliminary report just only 20 minutes.

The purpose of this study is to investigate the PCT during urinary tract infection, abnormal elevation of serum concentrations may be used as a biomarker in urinary tract infection and the severity of the diagnosis.

Method

This study collected patients diagnosed with urinary tract infection by physicians, regardless of age and gender, and perform urine culture and identification, urine routine examination and PCT test results from January 2013 to December 2015, to do follow-up data analysis comparison.

PCT test instrumentation is KRYPTOR compact PLUS. Instrument principle is formula homogeneous fluorescence immunoassay, that the signal intensity is proportional to the concentrations of the PCT. Serum samples were collected in SST test tubes for PCT test, and complete the test within 24 hours after harvest inspection.

The instruments we used for urine sediment test is Sysmex UF-1000i, that main principle is flow cytometry, a semiconductor laser as the light source. When the cells or particles through the flow cell, cells or particles are generated three kinds of light sources different messages which are fluorescence, forward scattered light, and side scattered light. Urine biochemistry tests using the automatic Urine Chemistry Analyzer, model AX-4030, the testing principle is Dual-wavelength reflectance method. Urine samples were collected in S-Y quantitative urine sediment tubes, specimen volume is 12mL.

We use three test items Bacteria, WBC esterase and sediment WBC of urine routine examination which help to diagnose UTI.

Urine culture specimen are collected in a sterile urine container, culture procedure should complete within 24 hours. A sufficient number of colonies and unpolluted cultured bacteria were used for the identification.

Figure & Table

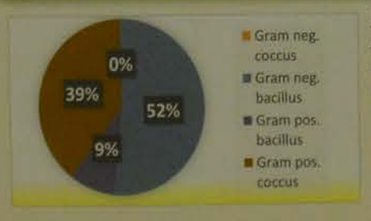


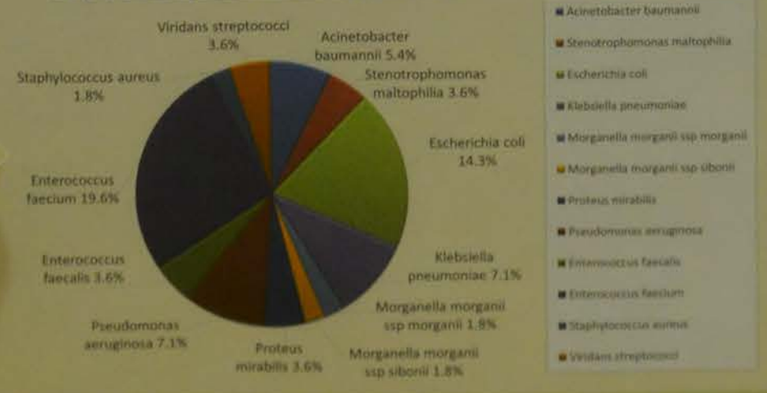
Figure 1.

42 patients' results were urine culture positive, according to Gram's stain classification were GNC 0%; GNB 52%; GPB 9%; GPC 39%

Figure 2.

The proportion of each species in positive urine culture results. Most of the top three are Enterococcus faecium, Escherichia coli, and Klebsiella pneumonia.

The proportion of positive urine culture of each strain



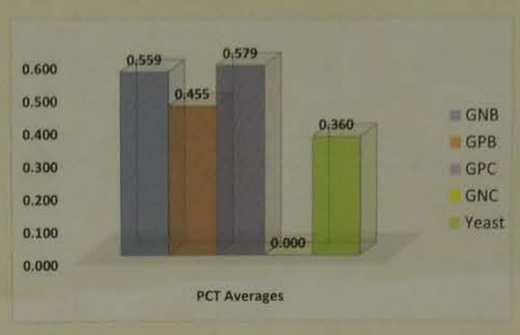


Figure 3.

Bar graph are based on Gram's stain to classify each species of positive urine culture. And the means of PCT in GNB, GPB, and GPC.
(No GNC culture positive results)

Table 1.

Urine culture results and three test items of urine routine examination : Bacteria, WBC esterase and sediment WBC compare the results with each other.

		Bacteria Negative	Bacteria Positive	Total	Bacteria Pos Rate	Bacteria Sensitivity	Bacteria Specificity	*LR(+)
Urine Culture	Positive	14	32	46	70%	70%	65%	2.0
	Negative	13	7	20	35%			
		WBC esterase Negative	**WBC esterase Positive**	**Total**	**WBC esterase Pos Rate**	**WBC esterase Sensitivity**	**WBC esterase Specificity**	**LR(+)**
Urine Culture	Positive	14	32	46	70%	70%	55%	1.6
	Negative	11	9	20	45%			
		WBC Negative	**WBC Positive**	**Total**	**WBC Pos Rate**	**WBC Sensitivity**	**WBC Specificity**	**LR(+)**
Urine Culture	Positive	13	33	46	72%	72%	35%	1.1
	Negative	7	13	20	65%			

*LR(+): positive likelihood ratio

Table 2.

Urine culture negative or positive, PCT results have significant differences. Coupled with the three of urine routine examination: Bacteria, WBC esterase and sediment WBC compared with each other, PCT results and urine culture positive results are more relevant.

	Urine culture Negative		Urine culture Positive	
	Bacteria Negative	Bacteria Positive	Bacteria Negative	Bacteria Positive
PCT mean	0.264	0.194	0.426	0.574
	WBC esterase Negative	WBC esterase Positive	WBC esterase Negative	WBC esterase Positive
PCT mean	0.243	0.235	0.654	0.546
	WBC Negative	WBC Positive	WBC Negative	WBC Positive
PCT mean	0.308	0.202	0.448	0.561

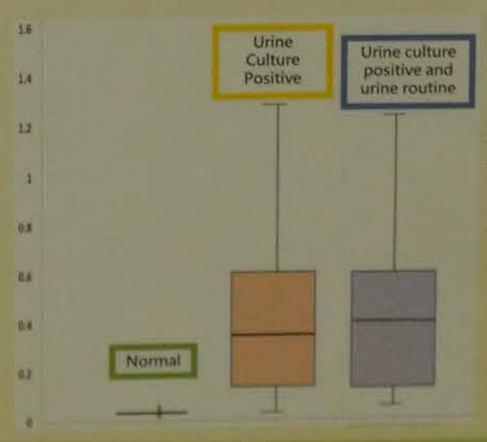


Figure 4.

- Normal : PCT < 0.05
- Urine culture positive : PCT median 0.352 (0.032 - 1.281)
- Urine culture positive and urine routine : PCT median 0.402 (0.058 - 1.234)

Result

62 patients was diagnosed as urinary tract infection (UTI) via doctors enroll in our study. Their ages range from 29 to 92 years old. Among the patients, 19 were males and 32 were females. A total of 42 patients had positive urine culture, and their serum PCT levels were 0.4±0.1ng/mL(Mean±SD), ranging from 0.06 to 1.25. In positive urine culture, Enterococcus faecium is the most(19.6%), and the others are Escherichia coli(14.3%) , Klebsiella pneumoniae(7.1%) , Pseudomonas aeruginosa(7.1%) and Acinetobacter baumannii(5.4%)(Fig. 2). Based on Gram's stain, GNB(52%) is the most of our patients, and the means of PCT in GNB, GPB, and GPC are 0.559ng/mL, 0.455ng/mL, and 0.579ng/mL, without GNC. (Fig. 3).

From statistics results of urine routine in our patients, Bacteria 1+ and above, sediment WBC over reference range and WBC esterase 1+ and above account for 28.92%, 33.73%, and 40.96%. Compared Bacteria, WBC and WBC esterase from Table 1., the sensitivity of Bacteria, WBC and WBC esterase in urine routine are 70%, but the specificity of Bacteria, WBC and WBC esterase in urine routine are low, especially WBC. When Bacteria and WBC esterase are positive, WBC is always positive. While WBC and WBC esterase are positive, Bacteria is not always positive. Compared Table 2. and PCT, PCT has significant differences between positive and negative urine culture, but has no significant differences between urine routine. Hence, PCT and positive urine culture have a correlation. In Fig 4, our patients are divided into three groups: healthy volunteers(PCT<0.05), patients with positive urine culture(PCT median 0.352, range from 0.032-1.281), patients with positive urine culture and positive of Bacteria, WBC and WBC esterase in urine routine(PCT median 0.402, range from 0.058-1.234). The latter two and healthy volunteers have significant differences, and the latter two have no significant differences. Hence, PCT can predict positive urine culture more accurately in early stage for UTI patients with urine routine.

Summary

In our study, PCT can predict whether the result of urine culture is positive or not, and evaluate positive urine culture more accurately for UTI patients with urine routine. Therefore, PCT can used to diagnose UTI by clinical doctor. The reference range of PCT is < 0.05ng/mL, and the mean of PCT with UTI is 0.4±0.1ng/mL(Mean±SD), obviously greater than 0.05 ng/mL. The detection time of PCT test takes 20 minutes, which is quicker than urine culture and has less external factors. Hence, we suggest that doctors can consider to order a PCT test when diagnosing UTI.

Immunology
PB-17

Immunology
PB-18

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PB-17

Evaluation of Mycoplasma pneumoniae IgA for Atypical Bacterial Pneumonia Diagnosis

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Introduction

Mycoplasma pneumoniae is a human pathogen that causes the pneumonia disease, a contagious respiratory infection. It is also a form of atypical bacterial pneumonia which occurs world-wide and becomes the most common cause of community-acquired pneumonia (CAP). In some cases, patient infected with M. pneumonia who has been related with illnesses, such as acute hepatitis, immune thrombocytopenic purpura, severe autoimmune hemolytic anemia, Stevens - Johnson syndrome, arthritis and transverse myelitis.

M. pneumoniae is one of the main causes of school-age children and young people. Its treatment is different from Streptococcus, Staphylococcus aureus, Haemophilus and other causes, because Panicillin and Cephalosporin are not inhibit M. pneumonia well in clinic. The clinical symptoms is hard to distinguished between M. pneumonia and other bacteria when patients are infected and contribute to pneumonia. Therefore, it is important to perform M. pneumoniae Antibody(Ab) test after patients get infected.

M. pneumoniae Ab IgG, IgM and IgA will rise in 7 to 14 days after infection. In the early phase of the disease, IgA rise quickly to peak levels and then decrease rapidly than IgM or IgG in a short time. IgA antibodies are more frequently found in case of primary infection in younger patients and reinfections. However, IgM are low or not found in elder patients, especially for reinfected ones. 10In this study, we collected serum specimens from M. pneumonia suspected patients performed with M. pneumonia IgG, IgM test in a Taiwan northern regional hospital, M. pneumonia IgA ELISA test then were performed to evaluate its benefit for M.pneumoniae diagnosis.

Materials and Methods

Serum sample

Seventy-five M. pneumonia suspected patients serum were collected, who already performed with M. pneumonia IgG, IgM ELISA test (DS2) and distributed to 3 groups (Table I). The first group was for those who had pulmonary infiltrates symptoms and confirmed by chest AP, the second group was the ones who were pneumonia suspected , and the third group was for the ones who were with similar symptom as pneumoniae or infected with other bacteria. All patients had tested with M. pneumoniae IgM, IgG ELISA serum test (DS2) in Taiwan northern regional hospital.

Mycoplasma pneumoniae IgA ELISA test

Diesse Chorus wasan automated systemand be designed to perform autoimmune and infectious disease tests by ELISA methods in ready-to-use single test device. The antigen composed of an extract of inactivated M. pneumoniaewas bound to the solid phase and is incubated with the human serum sample so that the specific IgA were bound to the antigen.

Results

- There were 17 samples in Group 1 (Table 2) who had diagnosed as pulmonary infiltrates with pneumoniae. Seventeen specimens got positive results with M. penumoniae IgA ELISA tests.
- Especially two young and three elder patients had negative results in M. pneumoniae IgG and IgM, but positive results in IgA. Those five patients had pneumoniae disease and belong to group 1.
- From Group 2 (Table 3), most of the patients were M. pneumoniae IgG positive, IgM negative and IgA equivocal result. It shows their development of immunity during pneumoniae infection.
- From Group 3 (Table 4), 48 patients had similar symptoms as pneumoniae, others had bacterial infection symptom in lung or bronchus. Their M. pneumoniae IgA test results were negative.

Conclusions

- M. pneumoniae IgA antibody plays an important role in acute-phase of M. pneumoniae diagnosis, it is a good indicator in young child, elderly and re-infected patients as some research before.
- M. pneumoniae IgA could be a tool not only for real-time diagnosis but also assist clinics to distinguish the pneumonia is infected with M. penumoniae or other pathogens.
- In the beginning of the M. pneumoniae infection, IgA shows borderline result to reflect patients' condition.

Table 1. Group Summary

	N	M. pneumoniae IgA	M. pneumoniae IgM	M. pneumoniae IgG
Group 1	17	Pos	Neg	Pos(12), Neg(5)
Group 2	10	Equ	Neg	Pos(9), Equ(1)
Group 3	48	Neg	Neg	Pos(22), Equ(4), Neg(22)

Pos: positive result, Neg: negative result, Equ:equivocal result.

Table 2. Serological results for M. pneumoniae in group 1

Gender	Age	M. pneumoniae IgA	M. pneumoniae IgM	M. pneumoniae IgG
Male	86	Pos	Neg	Pos
Male	67	Pos	Neg	Pos
Male	73	Pos	Neg	Pos
Male	37	Pos	Neg	Pos
Female	32	Pos	Neg	Pos
Female	29	Pos	Neg	Pos
Female	58	Pos	Neg	Pos
Male	38	Pos	Neg	Pos
Male	65	Pos	Neg	Neg
Male	71	Pos	Neg	Neg
Female	1	Pos	Neg	Neg
Male	3	Pos	Weak pos	Neg
Female	86	Pos	Neg	Neg
Female	4	Pos	Neg	Pos
Female	71	Pos	Neg	Pos
Male	4	Pos	Neg	Pos
Male	69	Pos	Neg	Pos

Table 3. Serological results for M. pneumoniae in group 2

Gender	Age	M. pneumoniae IgA	M. pneumoniae IgM	M. pneumoniae IgG
Male	89	Equ	Neg	Pos
Female	55	Equ	Neg	Pos
Male	33	Equ	Neg	Pos
Female	12	Equ	Neg	Pos
Female	52	Equ	Neg	Pos
Male	83	Equ	Neg	Pos
Male	88	Equ	Neg	Pos
Female	3	Equ	Neg	Equ
Male	88	Equ	Neg	Pos
Male	58	Equ	Neg	Pos

Table 4. Serological results for M. pneumoniae in group 3

Gender	Age	M. pneumoniae IgA	M. pneumoniae IgM	M. pneumoniae IgG
Female	44	Neg	Neg	Pos
Male	3	Neg	Neg	Pos
Male	70	Neg	Neg	Pos
Female	14	Neg	Neg	Pos
Male	9	Neg	Neg	Pos
Female	59	Neg	Neg	Pos
Female	6	Neg	Neg	Pos
Female	6	Neg	Neg	Pos
Male	6	Neg	Neg	Pos
Male	72	Neg	Neg	Pos
Male	53	Neg	Neg	Pos
Male	62	Neg	Neg	Pos
Female	73	Neg	Neg	Pos
Male	42	Neg	Neg	Pos
Female	12	Neg	Neg	Pos
Male	68	Neg	Neg	Pos
Male	1	Neg	Neg	Equ
Male	63	Neg	Neg	Neg
Female	60	Neg	Neg	Neg
Male	15	Neg	Neg	Pos
Female	60	Neg	Neg	Pos
Female	63	Neg	Neg	Equ
Female	50	Neg	Neg	Pos
Male	4	Neg	Neg	Neg
Female	6	Neg	Neg	Neg
Female	4	Neg	Neg	Neg
Female	4	Neg	Neg	Neg
Female	7	Neg	Neg	Pos
Female	33	Neg	Neg	Neg
Male	2	Neg	Neg	Neg
Female	4	Neg	Neg	Equ
Male	3	Neg	Neg	Neg
Female	3	Neg	Neg	Neg
Female	3	Neg	Neg	Neg
Female	1	Neg	Neg	Neg
Female	3	Neg	Neg	Neg
Female	4	Neg	Neg	Neg
Male	4	Neg	Neg	Neg
Female	1	Neg	Neg	Equ
Female	4	Neg	Neg	Pos
Male	2	Neg	Neg	Neg
Female	71	Neg	Neg	Neg
Male	59	Neg	Neg	Neg
Female	1	Neg	Neg	Neg
Male	5	Neg	Neg	Pos
Female	3	Neg	Neg	Neg
Female	58	Neg	Neg	Neg
Male	60	Neg	Neg	Neg

Immunology

PB-19

The frequency of the antibody for bovine protein in non-specific reaction of HTLV-1 antibody

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Introduction :

Antibody for bovine protein may cause the non-specific reaction of HTLV-1 antibody. However, the frequency is unknown.

Objective :

We demonstrate the non-specific reaction of HTLV-1 antibody by the assay of bovine serum albumin (BSA) IgG antibodies in the patient's serum, and the absorption test using adult bovine serum (ABS).

Methods :

We measured IgG BSA antibodies with the ELISA method using a BSA sensitization plate and peroxidase mark antihuman IgG antibody in the sera of 50 patients. The HTLV-1 antibody was measured by "LUMIPULSE G1200" (Fujirebio). The "SERODIA HTLV-1" (PA method : Fujirebio) was used for the confirmation of the non-specific reaction of HTLV-1 antibody. The absorption tests were as follows : 1) The 0.1mL of ABS was dispensed into a test tube, 2) The 0.1mL of the specimen was added into the tube, 3) The tube was mixed and incubated at 37 degrees Celsius for 2 hours, 4) The tube was centrifuged at 3000 rpm for 7 minutes, 5) It was measured the HTLV-1 antibody with the supernatant of the tube, 6) The phosphate-buffered saline was used instead of the control to the ABS, 7) The non-specific reaction was used as the absorption test less than half compared to the control.

Results :

We determined HTLV-1 antibodies in the 10,639 blood samples. The cases with PA method negative and LUMIPULSE positive (1 COI or more) were 102 cases (1%). The 50 blood samples of them were performed about absorption tests (age 0-38 years). Then 40 samples had non-specific reactions. The HTLV-1 antibody index of 40 samples as non-specific reaction were 2.2 ± 1.9 index (0-37 years old). And those of 10 samples as specific reaction were 2.5 ± 14 index (0-38 years old). There was no significant difference in the two groups (Fig.1,2). However, the BSA antibody index of 40 samples as non-specific reaction (19.2 ± 9.0 index) were significantly higher than those of 10 samples as specific reaction (8.4 ± 8.4 index) ($p = 0.0012$) (Fig.3,4). The result of overlapping 5 cases out of 40 cases that could be absorbed with the ABS, were shown in Table 1. The HTLV-1 antibody of 3 cases (case 3, 4, 5) remained positive but values of them were decreased. However, HCV antibodies became negative from positive (Table 1). Three cases of the 40 cases that could be absorbed with the ABS, were used to the absorption test using 30% BSA (Table 2). It could well absorb with ABS than BSA in the absorption tests.

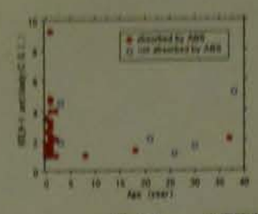

Fig. 1. Relevance of the HTLV-1 antibody values and age

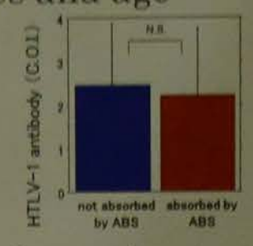

Fig. 2. ABS absorption test results and HTLV-1 antibody values

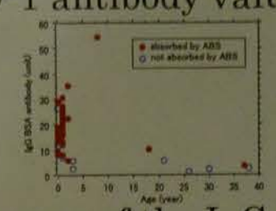

Fig. 3. Relevance of the IgG BSA antibody values and age

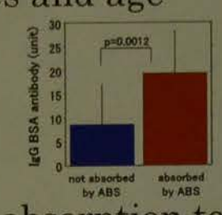

Fig. 4. ABS absorption test results and IgG BSA antibody values

Table1. 5 cases of duplication

HTLV-1 antibody (C.O.I.)	Absorption ratio (%)	IgG BSA antibody (unit)	HCV antibody (C.O.I.)	After blood collection (day)	Age (month)
Case 1					
1.8	72.7	18.7	1.6	0	7
4.0	65.7	24.2	2.6	83	9
4.6	95.2	23.6	3.1	91	10
3.3	87.5	26.1	1.5	151	12
Case 2					
3.4	47.6	11.5	4.9	0	2
3.5	70.4	13.2	4.0	89	5
4.8	82.1	24.0	4.9	427	16
Case 3					
9.2	89.0	17.0	3.8	0	9
2.1	91.7	35.2	0.3	301	16
Case 4					
3.2	82.4	19.4	1.1	0	12
2.2	83.3	16.3	0.8	18	12
Case 5					
1.4	75.0	16.6	1.4	0	4
1.1	83.3	13.1	0.3	207	11

Table2 Absorption test using 30% BSA

No	Absorption rate by ABS (%)	Absorption rate by BSA 30% (%)
1	88.9	-11.1
2	88.9	0.0
3	83.3	0.0

Discussion :

The reactivity of the difference due to the difference of the three-dimensional structure of the BSA itself may cause negative reduction in the absorption test by the BSA.

In our center, the reagent of HTLV-1 antibody has been changed to the new reagent from the old reagent in May 2011. Then, the cases of false positive suspected were increased. False positive rate of HTLV-1 antibody in this study was 1%. Number of cases that could not be absorbed by the ABS were 10 cases out of 50 cases, the false positive rate of unknown cause of HTLV-1 antibody was 0.2%. False positive rate in the former reagents has been reported to be 0.2%. The main cause of false positives increased by the improvement of the reagent is considered to be an antibody to the bovine protein.

Conclusion :

About 80% of non-specific reaction of HTLV-1 antibodies had antibodies derived from bovine protein in this study.

Immunology

PB-20

Comparison of the risks of tuberculosis infection among Japanese medical students and non-Japanese international students: tuberculosis screening using a T cell interferon-γ release assay

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Background: Screening of medical students and international students for tuberculosis (TB) at the time of admission is a key strategy to control and prevent the spread of infection on university campus and teaching hospitals because of the high risk of exposure to TB patients. The Mycobacterium tuberculosis antigen-specific interferon-γ release assays (IGRAs) are specific latent tuberculosis detection methods used in such groups.

Objective: To evaluate TB risk at the time of admission in university campus and medical schools in Japan, a retrospective study was conducted in 2009-2015.

Subjects and Methods: Between 2009 and 2015, a total of 2377 students (1730 Japanese students and 647 international students) were screened for TB using the IGRAs at the time of admission. QFT test (QuantiFERON-TB Gold In Tube assays) was carried out as IGRAs. QFT test was performed once at course entry. QFT test and analysis were performed according to the manufacturer's instructions.

Follow up of students who were QFT test positive at baseline: Students who were QFT test positive offered evaluation by infectious disease physician and their management including chest radiography, chest computed tomography and LTBI therapy.

Data analysis: Comparisons of proportions were tested for significance of difference using the $\chi 2$ test. For testing difference, $p = 0.05$ was used as a criterion for statistical significance.

Ethics: This study was approved by the Ethics Committee of Gunma University Graduate School of Medicine.

Results:

1. Positive ratio of QFT in students of medical school and international students of Gunma University

Only 0.3 % Japanese students were positive for IGRAs, but none were diagnosed with active TB at the follow-up. In contrast, 9.1 % international students were positive for IGRAs (table 1). Positive ratio of IGRAs in international students was significantly higher than that of medical students at the time of admission (figure 1).

2. Clinical breakdown of active TB during follow up in QFT positive students

Results are summarized table 2 and figure 2. QFT positive students (5 Japanese medical students and 59 international students) underwent chest x-ray and computed tomography and checked activities that might result in increased risk including volunteering, conduction research, mentoring, study abroad, traveling, visiting relatives, or employment involving close contact with individuals in areas with increased incidence of TB whether domestically or internationally. QFT positive five Japanese medical students had neither TB risk factors in past history, nor sign nor symptom of active tuberculosis disease during follow up. Two of 59 (3.4%) QFT positive international students were diagnosed with active tuberculosis disease by chest x-ray and computed tomography during follow up. Both students came from high-incidence countries of TB referring to World Health Organization (WHO). Other two treated by isoniazid in their own country. Another three came from low-incidence countries of TB defined by WHO. Two of 59 QFT positive international students, but not Japanese medical students, were diagnosed with active tuberculosis during follow up. These facts could be explained by over 94.1 % international students came from high-incident counties of TB referring to WHO (table 3). Positive ratio of QFT in Japanese medical students was significantly lower than that of international students.

Conclusion: Here we propose a standard approach for TB screening with IGRAs at the time of admission for medical students and international students in Japan.

Table 1. The results of QFT test among Japanese students and international students

		Japanese students n (%)	International students n (%)
Positive (≧ 0.35 IU/ml)	Male	1 (0.15%)	25 (7.1%) *
	Female	4 (0.37%)	34 (11.5%) *
	Total	5 (0.29%)	59 (9.1%) *
Intermediate (0.1 - 0.35 IU/ml)	Male	3 (0.46%)	17 (4.8%) *
	Female	10 (0.93%)	16 (5.4%) *
	Total	13 (0.75 %)	33 (5.1%) *
Negative (< 0.1 IU/ml)	Male	645 (99.4%)	309 (88.0%)
	Female	1067 (98.7%)	246 (83.1%)
	Total	1712 (99.0%)	555 (85.8 %)

Figure 1. Ratio (%) of QFT positive, intermediate, or negative versus total in Japanese medical students or international students of Gunma University in 2009-2015

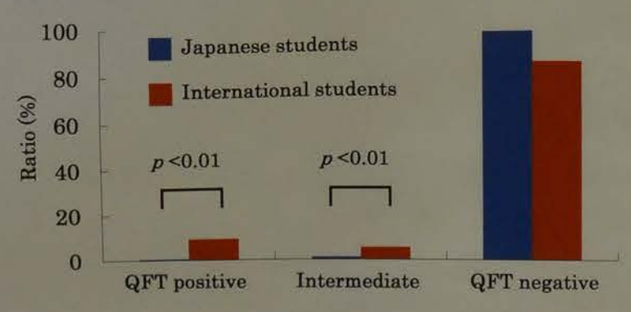


Table 3. QFT positive ratio among international students from "High Incidence "or "Low Incidence" countries of TB

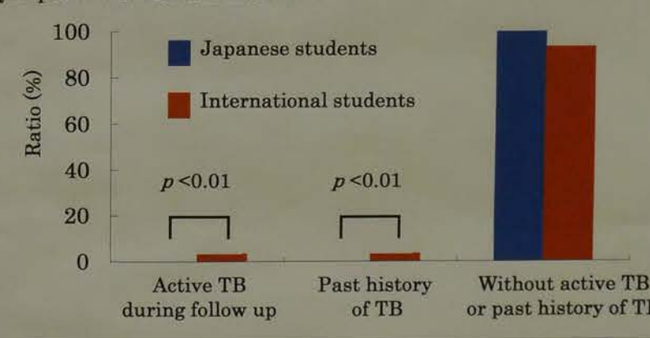
	n (%)	QFT positive ratio (%)
Total	647	9.1% (59/647)
High incidence countries of TB	609 (94.1%)	9.2% (56/609)
Low incidence countries of TB	38 (5.9%)	7.9% (3/38) *

* Three QFT positive subjects from TB low incidence countries were healthcare worker

Table 2. Ratio of active TB during follow-up or past history of TB in QFT positive students in 2009-2015

	Japanese medical students n (%)	International students n (%)
QFT positive	n = 5	n = 59
Active TB during follow-up	0 (0%)	2 (3.4%)
Past History of TB	0 (0%)	2 (3.4%)

Figure 2. Ratio (%) of active TB during follow-up or past history of TB in QFT positive students in 2009-2015



Immunology

PB-21

Impact of a five-year measles-rubella vaccination catch-up campaign in Japan: remarkable increase in seroprevalence of measles-specific immunoglobulin G among Japanese health care students.

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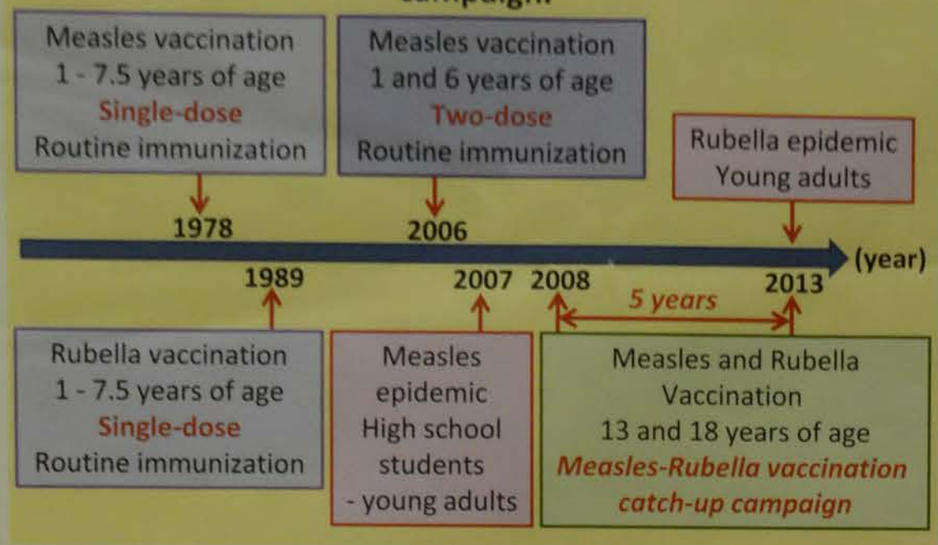
Background: In 2000, the United States declared that measles was eliminated from the United States. However, measles outbreak in 2014-2015 highlights a growing problem in the United States. In Japan, a measles epidemic occurred during the summer of 2007. In 2008, the Japanese government implemented a 5-year measles–rubella vaccination catch-up campaign for individuals aged 13 and 18 years old to eliminate measles until 2012 (fig. 1).

Figure 1. A 5-year measles-rubella vaccination catch-up campaign.



Objective: The present study was intended to determine the impact of the 5-year vaccination campaign by monitoring the seroprevalence of measles-specific and rubella-specific immunoglobulin (IgG) among Japanese healthcare students in 2007-2016.

Subjects and Methods: Between 2007 and 2016, serological screening of 2913 Japanese healthcare students at Gunma University (1069 males and 1844 females) for measles and rubella, was performed at the time of admission (table 1).
The 2913 subjects included
- Medical students: 1164 (40.0%)
- Nursing students: 875 (30.0%)
- Laboratory medical technology students: 445 (15.3%)
- Physical therapy students: 222 (7.6%)
- Occupational therapy students: 207(7.1%)

The subjects' characteristics are summarized in Table 1. Students with seronegative status were offered vaccination. Age, gender, and antibody titers were analyzed retrospectively.

Blood samples were collected from the healthcare students at the time of admission. Titers of anti-measles and anti-rubella antibodies were measured using an Enzygnost Enzyme-Linked Immuno-Sorbent Assay kit (Enzygnost® Anti-Measles-Virus/IgG, Enzygnost® Anti-Rubella-Virus/IgG, Enzygnost® respectively) provided by Siemens Healthcare Diagnostics K.K., Tokyo, Japan. Analyses were performed according to the manufacturers' instructions. Positive test results were defined as measles-specific IgG ≥300 mIU/ml and rubella-specific IgG ≥8 IU/ml. Antibody levels in the indeterminate range were considered seronegative (table 2).

Table 2. Cut-off value of Enzygnost® Anti-Measles-Virus/IgG and Enzygnost® Anti-Rubella-Virus/IgG.

	Quantitative assessment		
	Negative	Indeterminate	Positive
Anti-Measles Virus/IgG	< 150 mIU/mL	≥ 150 mIU/mL < 300 mIU/mL	≥ 300 mIU/mL
Anti-Rubella Virus/IgG	< 4 IU/mL	≥ 4 IU/mL < 8 IU/mL	≥ 8 IU/mL

Results:

1. The vaccination campaign caused a striking increase in the seroprevalence of measles-specific IgG, from 52.7% in 2007 to 96.6% in 2013. Japanese government declared that measles was eliminated from Japan in 2015. The seroprevalence remained > 90% during 2009–2016, except in 2014 (85.2%) and 2016(88.4%) (Fig. 2).

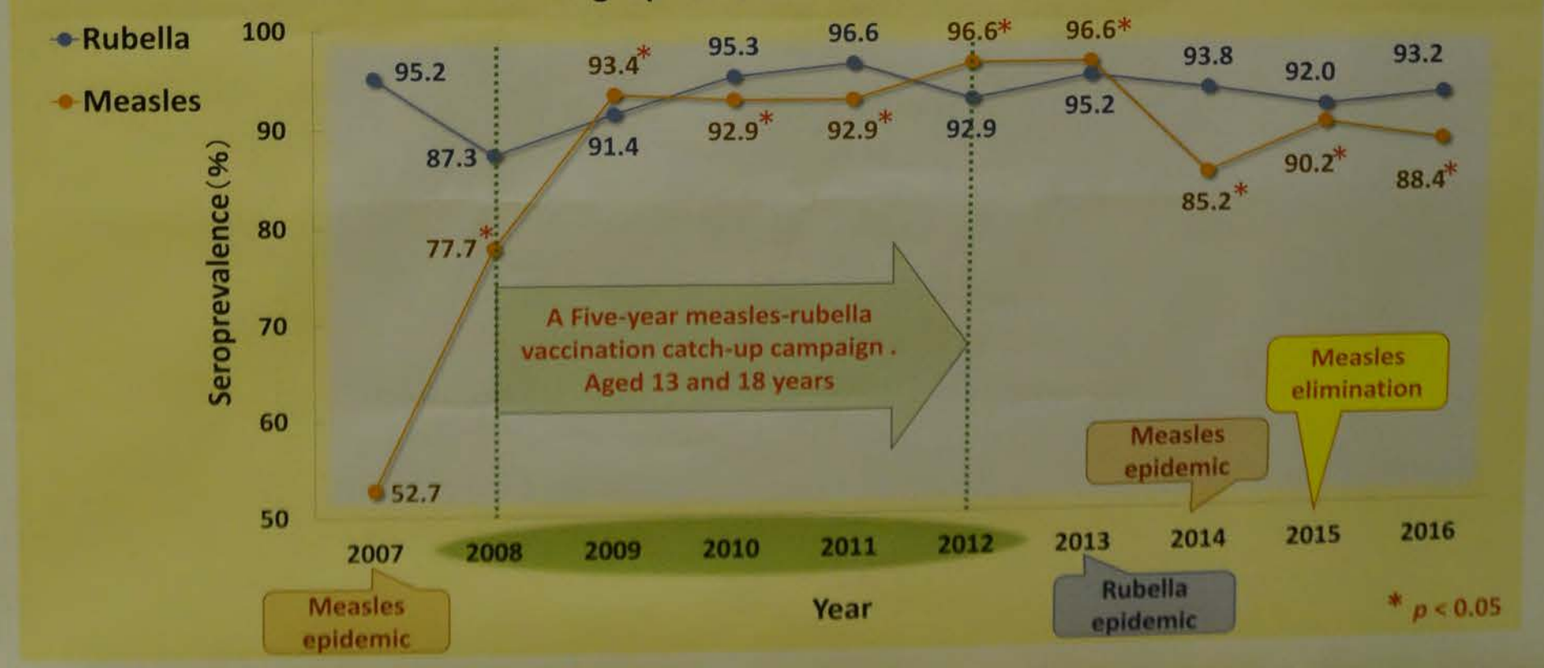
2. The seroprevalence of rubella antibodies remained >90%, except in 2008 (87.3%) (Fig. 2) .

Conclusion: The campaign raised the seroprevalence of measles-specific IgG by >50% during the 2007–2016 study period. In contrast, the measles–rubella vaccination campaign did not improve the protection against rubella because the seroprevalence was already >95% in 2007. Nonetheless, a substantial number of healthcare students remain susceptible to vaccine-preventable infectious diseases. Because their profession will involve frequent interactions with patients, including immigrants and travelers, we propose a nationwide targeted immunization program for Japanese healthcare students using serological screening prior to clinical training.

Table 1. Number, gender and age in health care students.

Year	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
Number	n = 273	n = 292	n = 302	n = 295	n = 294	n = 294	n = 293	n = 290	n = 287	n = 293
Gender										
Male/Female	82 / 191	107 / 185	104 / 198	81 / 214	106 / 188	114 / 180	124 / 169	114 / 176	108 / 179	129 / 164
Mean age, y±S.D.	19.9 ±2.3	19.9 ±2.1	19.9 ±2.2	19.9 ±2.0	19.9 ±2.1	19.9 ±2.2	19.0±2.5	19.1±3.1	19.2±3.3	19.2±3.4

Figure 2. Seroprevalence of measles/rubella-specific immunoglobulin G among Japanese healthcare students .



Immunology

PB-22

Novel base substitution in 5'UTR results in undetectable HCV RNA quantification by CAP/CTM Real-time PCR.

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Introduction.

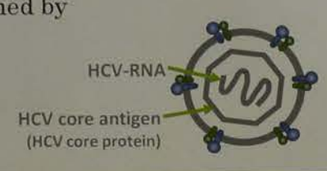
Accurate hepatitis C virus (HCV) quantification is essential for the diagnosis and the management of chronic hepatitis C therapy.

Real-time polymerase chain reaction (PCR) is widely used for highly sensitive assays for HCV RNA quantification. Two assays are widely used in many countries.

- AmpliPrep/Cobas TaqMan HCV assay (CAP/CTM) (Roche)
- Abbott Real Time HCV assay AccuGene m-HCV (Abbott)

The HCV core antigen assay is also an indicator of HCV infection. It is a test to detect the protein of the core particles that make up the virus particles. The results of HCV RNA and HCV core antigen are correlated with each other.

We describe here one patient infected with HCV genotype 2a in whom the CAP-CTM v1.0 assay repeatedly failed to detect HCV RNA, although hepatitis C viremia was confirmed by the HCV core antigen.



Sample.

Serum taken from 58-year-old man infected with HCV genotype 2a.

The HCV core antigen assay was positive results, however, TaqMan HCV assay v1.0 was undetectable.

TaqMan HCV assay v1.0 (Roche)	N.D.
HCV core Ag (Ortho-Clinical Diagnostics)	2287.80 fmol/l
HCV Ab (Abott)	11.9(+)

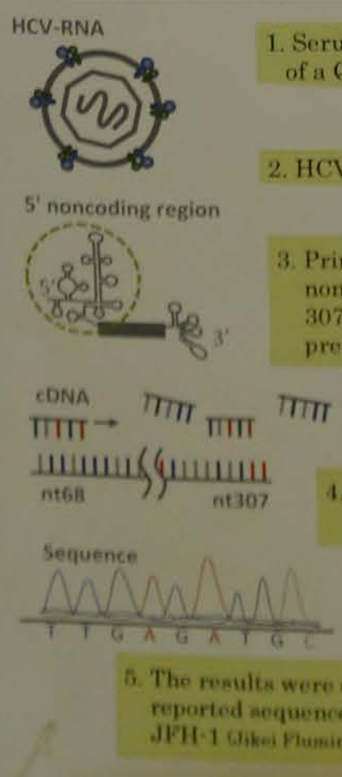
Methods.

Ⅰ. Additional HCV-related tests.

Serum HCV RNA quantification was performed by the Cobas AmpliPrep/Cobas TaqMan HCV assay (CAP/CTM) (Roche Molecular Systems) and Abbott Real Time HCV assay AccuGene m-HCV (Abbott Molecular, Inc). Serum HCV core Ag was performed by the HCV core Ag (Ortho-Clinical Diagnostics) and HCV core Ag (Abbott Molecular, Inc).

HCV RNA	TaqMan HCV assay v1.0 (Roche)
	TaqMan HCV assay v2.0 (Roche)
	AccuGene m-HCV (Abbott)
HCV core Ag	HCV core Ag (Ortho-Clinical Diagnostics)
	HCV core Ag (Abbott)

Ⅱ. Analysis of sequence in the HCV 5' noncoding region.



1. Serum RNA was extracted by means of a QIAamp viral RNA minikit.
2. HCV RNA was amplified by RT-PCR.
3. Primers to amplify the HCV 5' noncoding region between nt 68 and 307 were constructed based on the previous report. (1)
4. Direct sequencing was carried out using an ABI 310.
5. The results were compared with several previously reported sequences of HCV genotype 2a including JFH-1 (Jikei Fluminant Hepatitis 1) (2a). (1)(2)

Result.

Ⅰ. Additional HCV-related tests.

Serum HCV RNA was not detected by CAP/CTM v1.0, although hepatitis C viremia was confirmed by the AccuGene m-HCV (5.71 logIU/ml) and two kinds of the HCV core antigen assay (Abbott Diagnostic: 2079.49 fmol/l, Ortho-Clinical Diagnostics: 2287.8 fmol/l).

Recently CAP/CTM v2.0 with redesigned primers has been developed. HCV RNA measured by this assay was 5.8 logIU/ml, which is consistent with that measured by the AccuGene m-HCV.

We then investigated whether substitutions occur at the putative binding site of the TaqMan probe.

TaqMan HCV assay v1.0 (Roche)	N.D.
TaqMan HCV assay v2.0 (Roche)	5.80 logIU/ml
AccuGene m-HCV (Abbott)	5.71 logIU/ml
HCV core Ag (Ortho-Clinical Diagnostics)	2287.80 fmol/l
HCV core Ag (Abbott)	2079.49 fmol/l

Ⅱ. Analysis of sequence in the HCV 5' noncoding region.

In the putative binding site of the TaqMan probe, we observed two substitutions at position 144 (T to C) and position 147 (T to C), which is different from previously-reported substitutions (C1-C6).

These findings suggest that CAP/CTM v1.0 failed to detect HCV RNA in the patient due to the substitutions in the binding site of the TaqMan probe.

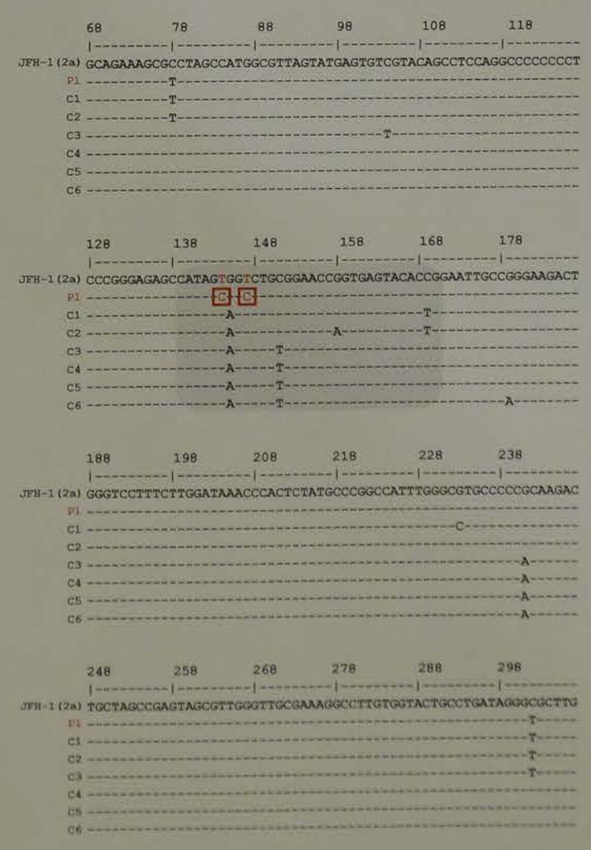


Fig1 P1: Sequence of patient.
C1-C6: Previously reported HCV RNA sequences undetected by CAP/CTM HCV version1.0. (1)(2)

Conclusion.

Previous studies reported that CAP/CTM HCV version1.0 failed to detect HCV RNA genotype 2a with substitutions at positions 145, 151, 158 and 169.
In this paper, we report the novel substitutions at position 144 and 147. These substitutions probably cause mismatches between the target sequences and the TaqMan probe.

The genetic tests provide excellent information on HCV diagnosis. Nevertheless, clinicians should be aware that CAP/CTM HCV version1.0 occasionally fails to detect HCV RNA genotype 2a and should consider alternative assays if there is a discrepancy between clinical findings and the laboratory results.

References.

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(2) Murilo,Y. 2014.Necessity for Reassessment of Patients with Serogroup 2 Hepatitis C Virus (HCV) and Undetectable Serum HCV RNA. Journal of Clinical Microbiology. 52 (2), 544-548.

Immunology

PB-23

Features and study on the use of Diapack3000 and MASTⅢ

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Introduction

Recently, the number of patients with I type allergic disease are increasing. The specific IgE testing is a basic diagnostic method for I type allergic disease. However, some of specific IgE testing have to use many serum, it is difficult to get serum from infants. In our laboratory, we utilize two analyzers for specific IgE, MASTⅢ and DiaPack3000. We can properly use these methods depending on the situations. We evaluated these analyzers, and compared each performance.

Method

From July 18 to December 15, 2015, 714 blood samples were processed with MASTⅢ.
First, we calculated the positive rate of each allergen. Next, we considered the correlation between MASTⅢ and DiaPack 3000, using 2 sample amounts - normal quantity (50/μL) and small quantity (30/μL). We tested for the top 5 allergens suggested by pediatricians , each produced a high positivity rate with MASTⅢ.

Analyzer and reagent

◆ MASTⅢ

◆ DiaPack3000 ORITONIgE[Chemiphar]

We summarized the 2method features and performance. (Table 1) In DiaPack3000, its measurement speed is 12 minutes and the items are selectable on demand. In MASTⅢ,it can measure 33items at same time and it uses 200μL by a test, however it takes about 6 hours.

Results

In MASTⅢ, The positive rates were as follows: inhaled allergens: 1.4%-58.1%, food allergens: 0.8%-14.7%, fruit allergens: 0.8%-1.7%.(Fig.1) The correlations of the 5 allergens that have highly positive rate (Japanese cedar pollen : T17, house dust mite : D2, cat dander : E1, egg white : F1, ovomucoid : F233) were 58.0–85.5% .(Fig.2) We also evaluated serum quantity of DiaPack3000, The correlation coefficient between normal quantity and less quantity samples were R=0.989-0.999.(Fig.3)

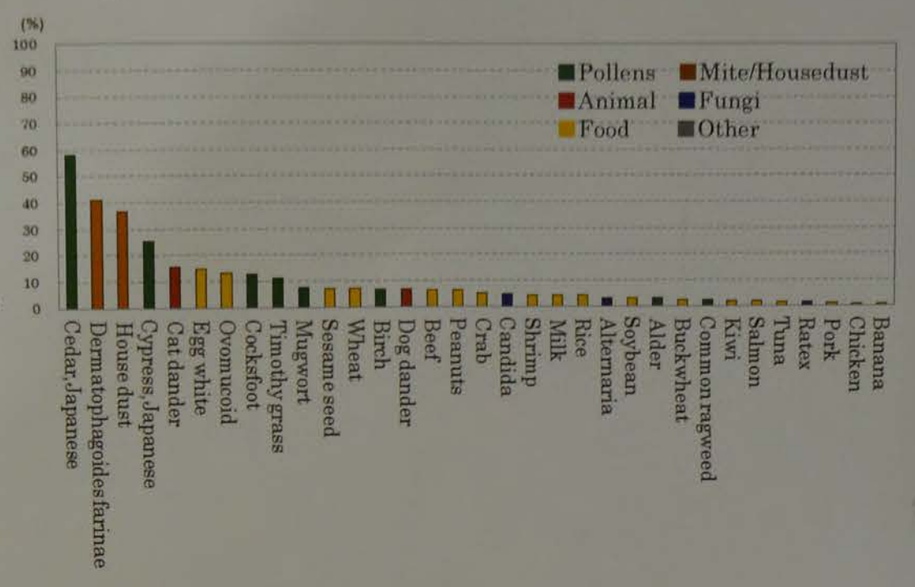
Conclusion

We found it is important that the allergy testing methods can use properly depending on the situations. We now understand purpose and characteristics of both testing method in specific IgE tests, and we are hopeful that these methods will continue to be used in diagnosis and treatment.

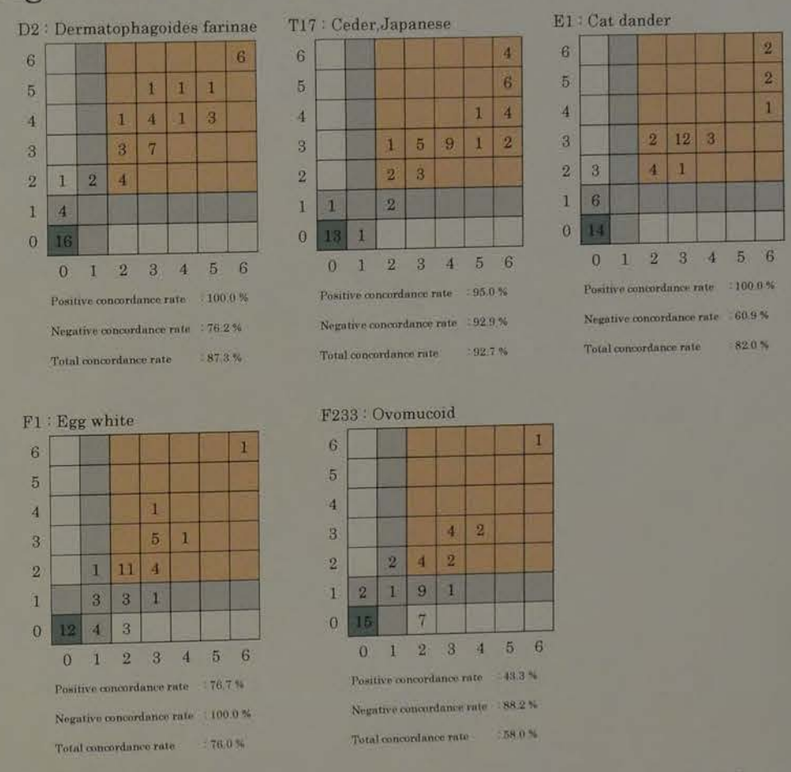
◆Table 1 Comparison

Analyzer	DiaPack3000	Lumimonster
Reagent	ORITON IgE [Chemiphar]	MASTⅢ
Principle of measurement	EIA	CLEIA
Scoring class	0 - 6	0 - 6
Allergen reagent property	Liquid phase	Solid phase
Sample quantity	50 μL / test	200μL / sample (33 test)
Measurement time	12 minutes	6 hours
Measurement interval	18 seconds	6 hours
Capacity (test / min)	90 tests / 30 minutes	5samples / 1measurement
Utilization	Inspection in accordance with needs	Multi-item simultaneous screening

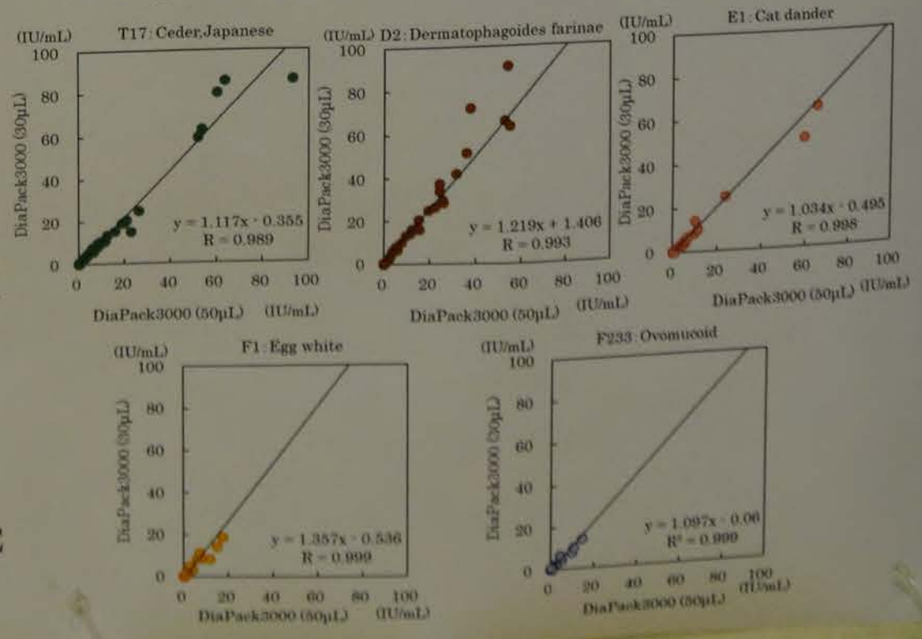
◆Fig.1 The Positive rate of MASTⅢ



◆Fig.2 Correlation of 2method



◆Fig.3 Correlation of normal quantity and small quantity



Immunology

PB-24

Evaluation of serum MMP-3 in HTLV-1–antibody-positive patients with rheumatoid arthritis

Kaori Ishihara[1]、Tetsuya Usui[1]、Norihito Kaku[1,2]、Syouichi Fukui[3]、Souichirou Minami[1]、Hiroo Hasegawa[1,2]、Atsushi Kawakami[3]、Katsunori Yanagihara[1,2]

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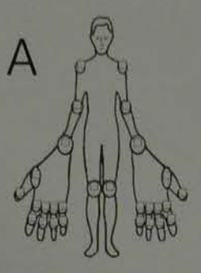
Introduction

Matrix metalloproteinase-3 (MMP-3) is an enzyme, which plays a role in the destruction of cartilage and bone in rheumatoid arthritis (RA). Several studies suggested that serum MMP-3 reflects synovial inflammation. However, there was no study on serum MMP-3 levels in the Human T Lymphotropic Virus Type I (HTLV-1) antibody-positive patients with RA (HTLV-1-pos-RA). Since serum C-reactive protein (CRP) levels and responses to the treatment were different between two groups, our study we compared serum MMP-3 levels between HTLV-1-pos-RA and HTLV-1 antibody-negative patients with RA (HTLV-1-neg-RA).

Methods

Twenty patients with RA (10 cases, HTLV-1-pos; 10 cases, HTLV-1-neg) who obtained the informed consent about clinical research from 2009 June to 2015 June were enrolled in this study. The serum MMP-3 levels, disease activity score 28 joints (DAS28)-CRP and DAS28–ESR(Fig. 1) were evaluated at both pre- and post-treatment.

A



B

Disease activity	DAS28CRP	DAS28ESR
High	4.1<	5.1<
Moderate	2.7～4.1	3.2～5.1
Low	<2.7	<3.2
Remission	<2.3	<2.6

Fig. 1 Evaluation of disease activity of RA
A the evaluated joints in DAS28
B The relationships between disease activity and DAS28.

Results

At the pre-treatment, the mean MMP-3 level in HTLV-1-pos-RA were lower than that in HTLV-1-neg-RA (174.8 ng/ml ±199 ng/ml vs. 234.4±262, P＝0.32). The mean CRP levels in HTLV-1-pos-RA were also lower than that in HTLV-1-neg-RA (1.15±0.94 mg/dl vs. 2.81 mg/dl±2.24、P=0.07) (Table. 1).

Table. 1 At pre- and post-treatment, the means , the SDs and P values of CRP, ESR,DAS28CRP, DAS28ESR and MMP-3 in HTLV-1(+) and HTLV-1(-) RA patients

Pre-Treatment	HTLV-1 (+)	HTLV-1 (-)	P value
CRP	1.15 ±0.94	2.81 ±2.24	0.07
ESR	48 ±33	51 ±28	0.43
DAS28CRP	5.4 ±0.9	5.1 ±1.6	0.34
DAS28ESR	6.2 ±1.2	5.6 ±1.7	0.28
MMP-3	174.8 ±199	234.4 ±262	0.32

Post-Treatment	HTLV-1 (+)	HTLV-1 (-)	P value
CRP	0.18 ±0.23	0.23 ±0.27	0.32
ESR	22 ±14	14 ±12	0.11
DAS28CRP	1.9 ±0.7	2.7 ±1.2	0.07
DAS28ESR	2.7 ±0.9	3.1 ±1.4	0.27
MMP-3	77.4 ±48	77.2 ±65	0.50

In HTLV-1-pos-RA, DAS 28 and MMP-3 levels at post-treatment were significantly lower than those at pre-treatments (DAS28CRP, P<0.05; DAS28ESR, P<0.05; MMP-3, P<0.05). (Fig.2A) Although DAS28 at post-treatment were significantly lower than those at pre-treatment in HTLV-1-neg-RA (DAS28CRP, P<0.05; DAS28ESR, P<0.05), there was no significant change in MMP-3 level (P=0.06)(Fig. 2B). In addition, there is no correlation between MMP-3 level and DAS28 in both HTLV-1-pos-RA and HTLV-1-neg RA(data not shown).

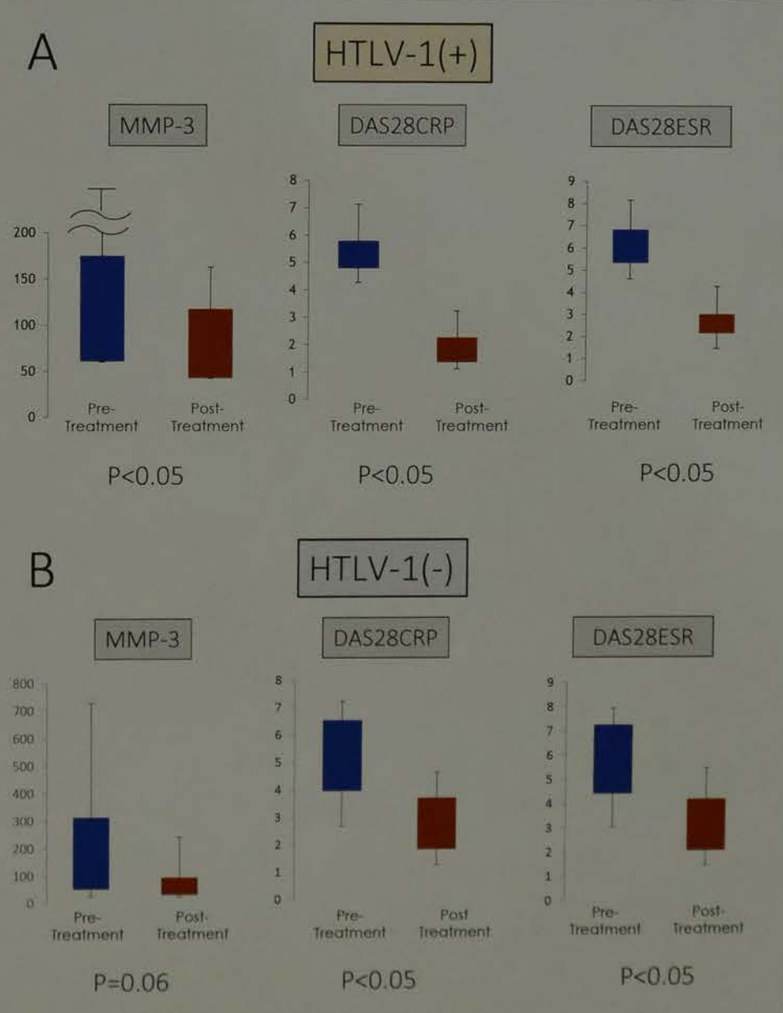


Fig. 2 MMP-3, DAS28CRP and DAS28ESR levels at pre- and post-treatments in HTLV-1-pos(A) -and HTLV-neg(B)-RA
In HTLV-1-pos-RA, decrease in DAS 28 and MMP-3 levels between pre- and post-treatment were significant(A), and in HTLV-1-neg-RA, only the difference of MMP-3 levels was not significant.

Conclusion

In HTLV-1-pos-RA, DAS28 as well as MMP-3 were significantly decreased by the treatment. However, there were no correlation between MMP-3 level and DAS28. Further study is needed to determine the usefulness of serum MMP-3 in HTLV-1-pos-RA.

Immunology

PB-25

The evaluation of fundamental performance of HISCL-5000® for the measurement of serum NT-proBNP.

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【Abstract】

N-terminal pro B-type natriuretic peptide (NT-proBNP) is a useful biomarker for the diagnosis and/or management of chronic heart failure. Here, we show the results of evaluation of a new instrument for serum NT-proBNP assay, HISCL-5000® (sysmex). We performed fundamental evaluation of the NT-proBNP assay using HISCL-5000®, intra and inter-assay variance, linearity for the serial dilution samples and influence of several substances. Coefficients of variance (CV) for intra and inter assays using control samples were between 0.9 and 2.4%. In a linear regression analysis for the serial dilution samples (0 - 34,434pg/mL), we obtained coefficient of linearity of 0.994. There is no significant interference, less than 10%, by existence of direct bilirubin, conjugated bilirubin, hemoglobin, chylous fluids, and rheumatoid factor into the measurements of the NT-proBNP. Furthermore, we evaluated a consistency of this assay with another electro-chemiluminescence immunoassay for NT-proBNP (Eclusys® analyzer, Roche Diagnostics KK, Tokyo, Japan) using 112 serum samples. Although the absolute values for HISCL-5000® measurements tend to be lower compared with those for ECLusys® measurements (the linear regression equation: y = 0.85x + 183.2), obtained absolute values were considered consistent with each other (correlation coefficient of 0.99).
These results indicate that HISCL-5000® can be applied for the serum NT-proBNP measurements in routine use for the laboratory.

【Backgrounds】

1. Pro B-type natriuretic peptide (proBNP) is produced in the myocardium, and is cleaved into N-terminal pro BNP (NT-proBNP) and the biologically active BNP.
2. NT-proBNP is well known as a useful biomarker for the diagnosis and/or management of chronic heart failure.

【Aim】

To know the clinical usefulness of a new instrument for the measurement of serum NT-proBNP, HISCL-5000®, we evaluated fundamental performance of HISCL-5000® for the measurement of serum NT-proBNP.

【Results】

Intra and inter assay variations

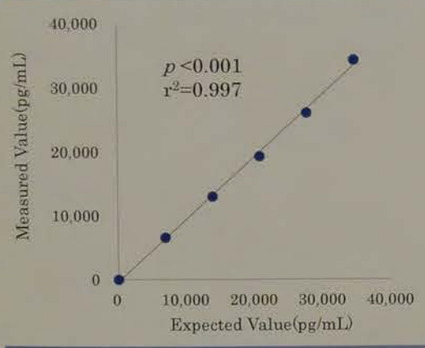
Intra-assay variation

	Control	
	Low	High
Mean (pg/mL)	141.6	4412.7
SD	1.73	41.39
CV(%)	1.2	0.9

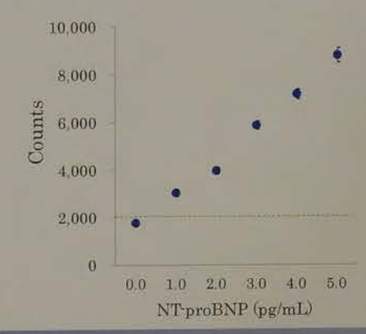
Inter-assay variation

	Control	
	Low	High
Mean (pg/mL)	143.5	4444.4
SD	3.49	88.67
CV(%)	2.4	2.0

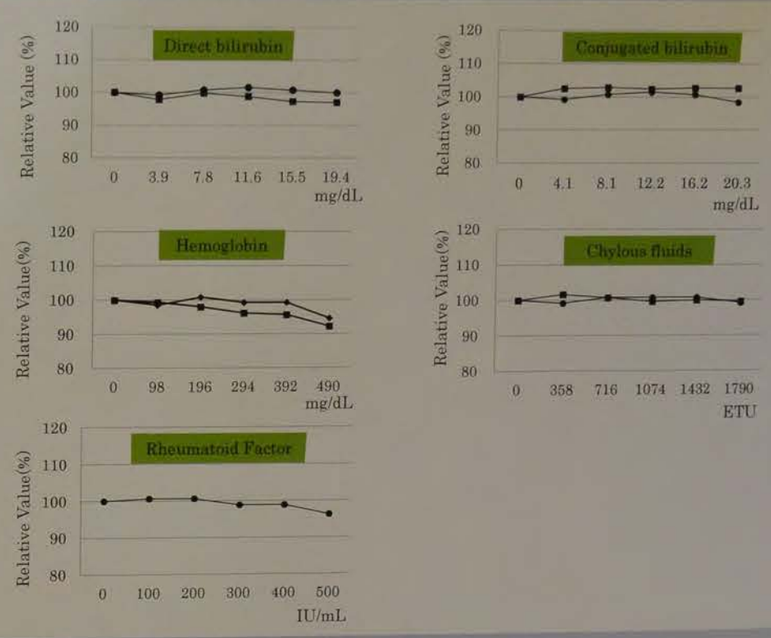
linearity



Detectable limit



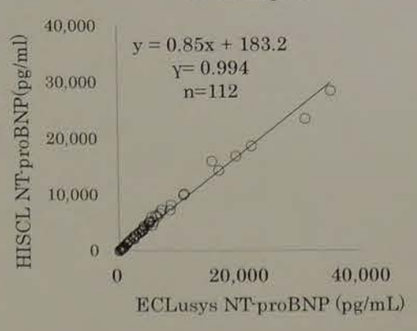
Influences of reference substances



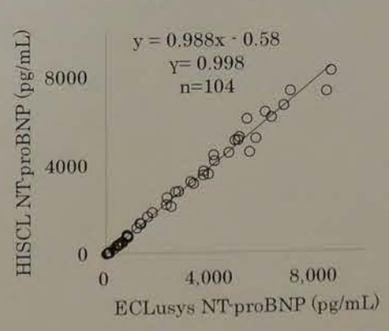
Comparison between HISCL® NT-proBNP assay and ECLusis® NT-proBNP

Correlation of absolute values between the two methods

All samples



NT-proBNP < 10,000 pg/mL.



Clinical classification of chronic heart failure based on serum NT in 112 samples

HISCL-5000®	ECLusys® NT-proBNP pg/mL	<125	125–399	400–899	900–3,999	4,000–7,999	≥8,000
NT-proBNP pg/mL	class	1	2	3	4	5	6
<125	1	81					
125–399	2		1	2			
400–899	3			10			
900–3,999	4				15		
4,000–7,999	5					12	1
≥8,000	6						10

【Summary】

1) Intra and inter assay variations: Coefficients of variance (CV) using control samples were between 0.9 and 2.4 %.
2) linearity for the serial dilution samples: In a linear regression analysis for the serial dilution samples, constant linearity was observed between 0 and 34,434pg/mL ($r^2 = 0.997$, $p < 0.001$).
3) Detectable limit: Detectable limit of serum NT-proBNP in HISCL-5000® was determined as 1.0 pg/mL.
4) Influences of reference substances: There is no significant interference, less than 10%, by existence of direct bilirubin, conjugated bilirubin, hemoglobin, chylous fluids, and rheumatoid factor into the measurements of the NT-proBNP.
5) Comparison of HISCL® NT-proBNP assay with another NT-proBNP assay: We compared the NT-proBNP values measured by this assay with those measured by an electro-chemiluminescence immunoassay (Eclusys® analyzer, Roche Diagnostics KK, Tokyo, Japan) using 112 serum samples. Obtained absolute values were considered consistent with each other (correlation coefficient of 0.99), although we observed a better consistency (the linear regression equation: y = 0.99x +-0.58) in samples with NT-proBNP concentrations < 10,000 pg/mL.

【Conclusion】

These results indicate that HISCL-5000® can be applied for the serum NT-proBNP measurements in routine use for the laboratory.

Immunology

PB-26

Analytical Performance of the i-STAT Cardiac Troponin I Assay

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[*1] Department of Clinical Laboratory, Toho University Omori Medical Center
[*2] Department of Laboratory Medicine, Toho University School of Medicine

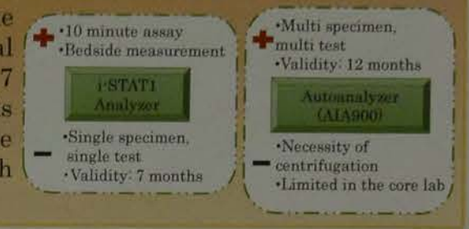
Background

Our central laboratory requires 30 minutes from the arrival of the specimen, to reporting the result of cardiac troponin I (cTnI). Although 60 minutes of turn around time (TAT) is desirable, delays occur in the collection and transportation of the blood. This delay may cause latency to diagnose myocardial infarction. In emergency departments, POC tests are used to meet the goal. The i-STAT1 Analyzer (Abbott Point-of-Care) is one of the POC tests widely used in many emergency departments.

The purpose of this evaluation is to check basic analytical performances such as its precision, inter-day CV(%), linearity, detection limit, interferences, followed by comparison to the AIA900 (Tosoh).

Conclusion

The basic analytical performances for the i-STAT1 Analyzer were satisfactory. The i-STAT has the disadvantage of measuring only one specimen at a time, which might cause delays when several specimens arrive at once. The term of validity for i-STAT cTnI cartridge is short, for about 7 month, which is almost half of the term, when compared to AIA900. However, since i-STAT is portable and can provide immediate results in just 10 minutes, the use of i-STAT would be suitable in emergency departments rather than central laboratories. It should also be utilized in areas with limited access to power and water supplies.



Result

1. ***Precision***: Three levels of lithium heparinized plasma, pooled according to the cTnI concentration, were assayed ten times.
2. ***Lot-to-lot imprecision***: Fifty of the randomly selected lithium heparinized specimens were tested for two different lots, at concentrations ranging from 0.00 to 35.01 ng/mL.
3. ***Inter-day CV(%)***: Three levels of controls were assayed twice a day (10AM, 4PM), for ten days.
4. ***Upper limit of linearity***: Heparinized plasma samples containing high concentrations of cTnI were serially diluted with samples non-containing cTnI, and assayed.
5. ***Limit of detection***: Blank sample was measured in ten replicates, and ten low level samples were measured in five replicates each.
6. ***Interference***: Chyle (14500 FTU), Hemolytic hemoglobin (4900 mg/dL), Bilirubin F (195 mg/dL), Bilirubin C (209 mg/dL) were added to samples adjusted to cTnI 0.10 ng/mL, and serially diluted, and assayed.
7. ***Correlation***: 150 patients with the request of troponin analysis were tested by two applications, the i-STAT and AIA900.
8. ***Concordance***: Five discrepancies were retested by both applications.

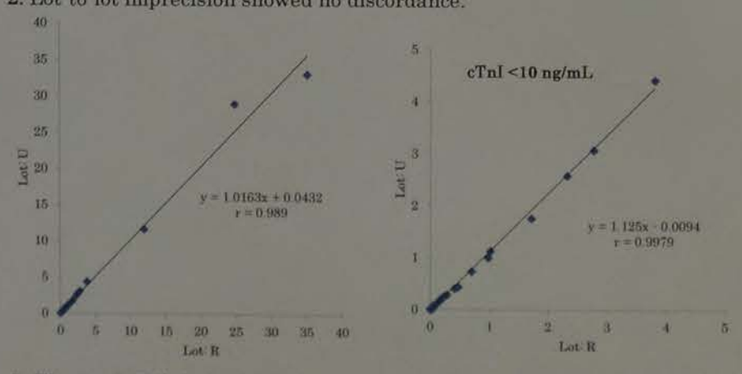
*The protocol for this investigation was approved by the ethics committee

1. The total precision was satisfactory with CV(%) of 5.56 - 8.35 %.

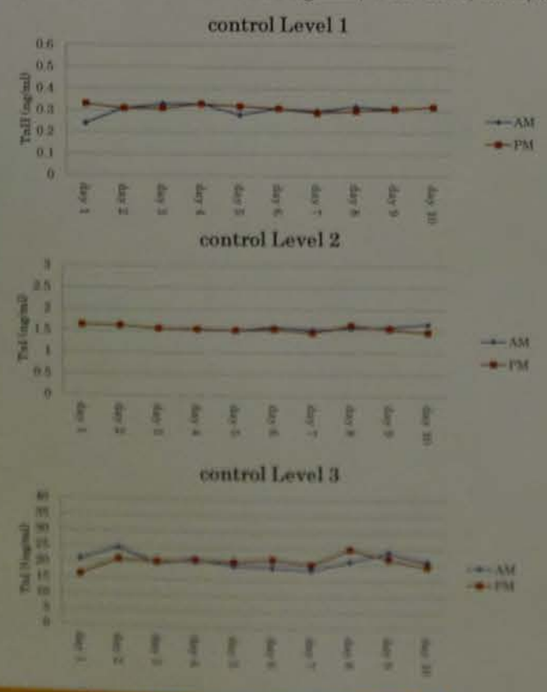
	cTnI concentration		
	low	medium	high
1	0.10	23.42	49.96
2	0.09	30.70	50.00
3	0.10	29.56	49.40
4	0.09	31.20	40.44
5	0.10	28.98	41.03
6	0.09	30.66	50.00
7	0.09	29.57	50.00
8	0.10	27.94	43.91
9	0.10	28.73	50.00
10	0.09	29.24	46.19

	cTnI concentration		
	low	medium	high
average	0.095	29.00	47.09
SD	5.27x10^-3	2.20	3.93
CV(%)	5.55	7.58	8.35

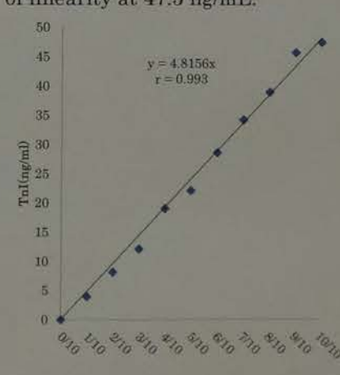
2. Lot-to-lot imprecision showed no discordance.



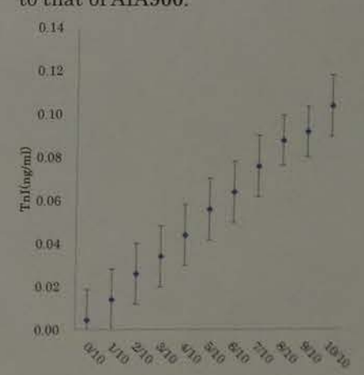
3. The total CV(%) obtained for control 1 (0.24–0.44 ng/mL), control 2 (1.14–2.12 ng/mL), and control 3 (11.98–31.58 ng/mL), were 6.8%, 3.6%, and 9.7%.



4. The i-STAT cTnI assay demonstrated an upper limit of linearity at 47.5 ng/mL.



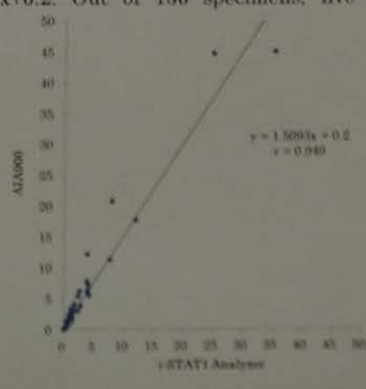
5. The detection limit of the i-STAT was 0.03 ng/mL, which was similar to that of AIA900.



6. The assay was not affected by common interferences.

	Common Interferences			
	Chyle	Hemoglobin	Bilirubin F	Bilirubin C
0/10	0.09	0.07	0.08	0.08
1/10	0.08	0.08	0.08	0.09
2/10	0.08	0.07	0.08	0.10
3/10	0.09	0.08	0.06	0.10
4/10	0.08	0.08	0.08	0.09
5/10	0.08	0.08	0.06	0.08
6/10	0.09	0.08	0.07	0.08
7/10	0.09	0.08	0.06	0.09
8/10	0.09	0.08	0.06	0.09
9/10	0.09	0.08	0.08	0.08
10/10	0.09	0.09	0.07	0.07

7. The observed correlation coefficient between i-STAT and AIA900 was r=0.95, y=1.509x+0.2. Out of 150 specimens, five specimens observed discrepancy.



8. Out of the five discrepancies, two became negative, and two remained positive after the retest. Cutoffs used were based on the 99th percentile values for the i-STAT (0.08 ng/ml), and clinical cutoff in our laboratory for the AIA900 (0.10 ng/ml).
*SS: short sample

SEQ	i-STAT	AIA900
28	–	+
31	–	+
46	–	+
76	–	+
85	–	+

→

SEQ	i-STAT	AIA900
28	–	–
31	–	–
46	SS*	SS*
76	–	+
85	–	+

i-STAT1 Analyzer (Abbott Point-of-Care)

<Benefits>

The i-STAT is a handheld POC test (weight 650 g), which analyzes various blood tests using whole blood or plasma. Bedside measurements can reduce the time for transportation and centrifugation, which leads to the shortening of TAT.

The manipulation process using a compact plastic disposable cartridge is clear and simple, which enables nurses and doctors perform the assay easily and reliably.

The i-STAT automatically saves power when it is not used for 3 minutes. With this ability, i-STAT could be used for several weeks without charging the battery.

<i-STAT cartridges>

- Cardiac Markers (cTnI, BNP, CK-MB)
- Chemistries (Na, K, Cl, TCO2, Anion gap, Ca, Glucose, UN, Crea, Lactate)
- Hematology (Hct, Hgb)
- Blood Gas (pH, pCO2, pO2, TCO2, HCO3, BE, sO2)
- Coagulation (ACT Kaolin, ACT Celite, PT/INR)

Immunology

PB-27

Detection of the anti Stress Granule antibody using U2OS

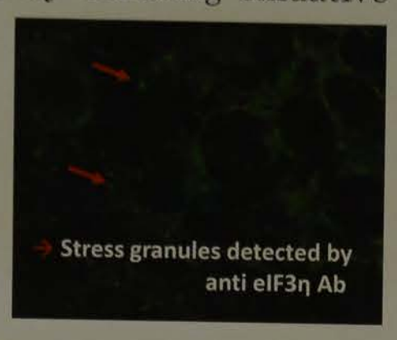
Takayuki MORIMOTO , Satoshi YAMASAKI, Yusuke YOSHIDA, Michiya YOKOZAKI, Eiji SUGIYAMA

Hiroshima University Hospital

Background: Examination of autoantibody is important for making diagnosis or evaluating disease activity of autoimmune diseases. Therefore, establishment of a method to detect new autoantibody contribute to a better diagnosis and prediction of prognosis of autoimmune diseases.

Sodium arsenite is known to induce cytoplasmic formations of RNA granule named stress granule by inducing oxidative stress in a cell.

In this study, we exa-mined the frequency of anti stress granule antibody (anti SG ab) in patients who visited Rheumatology Clinic in our hospital.



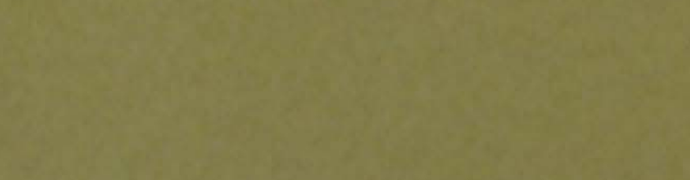
Methods: We treated U2OS cell, an osteosarcoma cell line, with sodium arsenite to induce stress granule (SG) in the cell. We applied an indirect immunofluorescence technique by using antibody against eIF3η, a marker protein for SG, to detect anti SG antibody in patient's sera.

0.2million U2OS cell/well
on cover slip in 24 well plate
↓ O/N
500 μ M sodium arsenite
↓ 60 min
fix by 4% paraformaldehyde
↓ 15 min
100% methanol at −20℃
↓ 5 min
70% ethanol at 4℃
↓ > 4hours
immunostaining

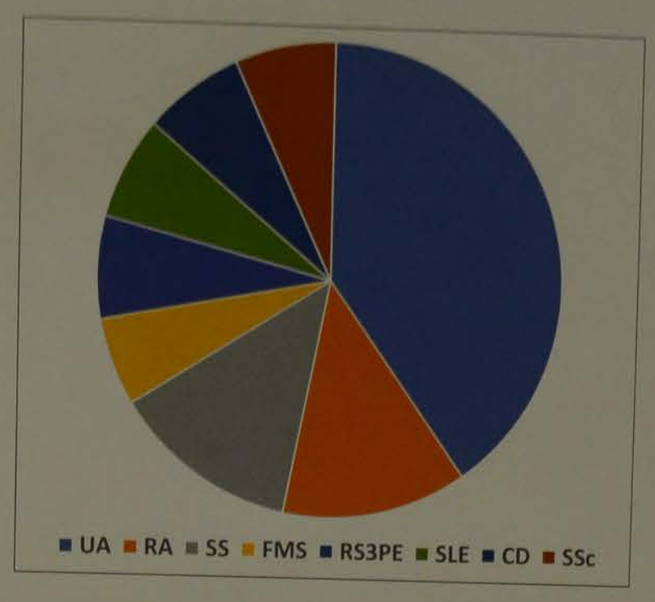
blocking fixed cell by
5% horse serum in PBS
↓ 60 min
anti eIF3h ab & patient serum
↓ 60 min
wash by PBS
↓
Cy2 labelled anti goat ab
Cy3 labelled anti human ab
↓ 60 min
wash by PBS
↓
mount and observe

Results: Among 120 cases examined, 17 samples (14.2%) were positive for anti SG antibody. None of those samples were positive for anti cytoplasmic antibody by FLUORO HEPANA TEST.

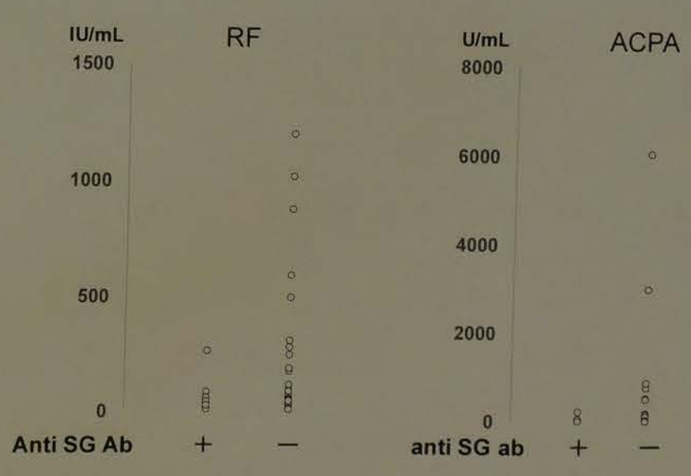
A representative case with anti SG ab



a : Detexction of SG by anti eIF3η antibody
b : patient's serum
c : merge of a) & b)



The anti SG antibody positive cases include 6 cases of undifferentiated arthritis (UA), 3 case of rheumatoid arthritis (RA), 1 case of Sjögren's syndrome (SS), Fibromyalgia (FM), Remitting Seronegative Symmetrical Synovitis With Pitting Edema (RS3PE), systemic lupus erythematosus (SLE), Crohn's disease (CD), and systemic sclerosis (SSc). All the cases except one had arthralgia as a chief complaint when they visited our clinic.



There were 87 cases with joint pain among 120 included. Among these population, the titer of Rheumatoid factor (RF) and anti-citrullinated protein antibody (ACPA) were lower in patients with anti SG antibody compared to those without anti SG antibody.

Conclusion: It is suggested that testing anti SG antibody can be used to making a diagnosis of autoimmune arthritis in undifferentiated arthritis cases. The anti SG antibody is also positive in several autoimmune diseases.

Immunology

PB-28

Comparison of PIVKA-II Assay Performances -CLEIA and CLIA principles-

Eri Ishii

Kurashiki Medical Center

1.Study Design

We evaluated the analytical performances of recently developed Architect PIVKA-II (hereinafter "Architect") by Abbott Japan and STACIA PIVKA-II (hereinafter "STACIA") by EIDIA, the former of which basing on CLIA and the latter of which basing on CLEIA principle in terms of reproducibility, limit of detection, linearity and correlation. For the evaluation of correlation, we employed Picolumi PIVKA-II MONO(hereinafter "Picolumi")) as the reference method. The study was performed upon approval from ethics committee in our hospital.

Product	Architect·PIVKA-II	EIDIA STACIA PIVKA-II	PIVKA-II MONO
Manufacturer	Abbott Japan	EIDIA	EIDIA
Principle	CLIA	CLEIA	ECLIA
Instrument	ARCHITECT	STACIA	Picolumi
Assay time	29mins.	13.3mins.	19mins.
Cut-off value	40mAU/mL	40mAU/mL	40mAU/mL
Assay range	5.06-30,000mAU/mL	10-75,000mAU/mL	10-75,000mAU/mL
Sample type	Serum・Plasma	Serum・Plasma	Serum・Plasma
Capture Ab*1	3C10	MU-3	MU-3

*1 3C10 antibody has been reported to recognize the equivalent epitope as MU-3 and has equivalent reactivity with PIVKA-II as MU-3 does (Clinical Biochem 2015:48;1120–1125).

2.Results

1) Within-run Reproducibility (n=10)

Architect	pool serum	
	1	2
Mean (mAU/mL)	30.9	98.5
SD	2.0	5.0
CV(%)	6.4	5.1

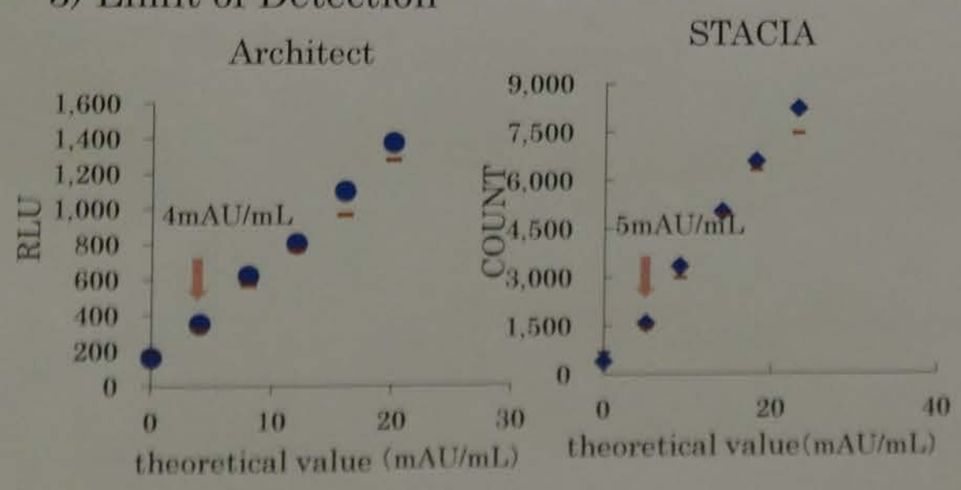
STACIA	pool serum	
	1	2
Mean (mAU/mL)	31.6	109.2
SD	1.5	5.2
CV(%)	4.8	4.7

2) Day-to-day reproducibility (n=10)

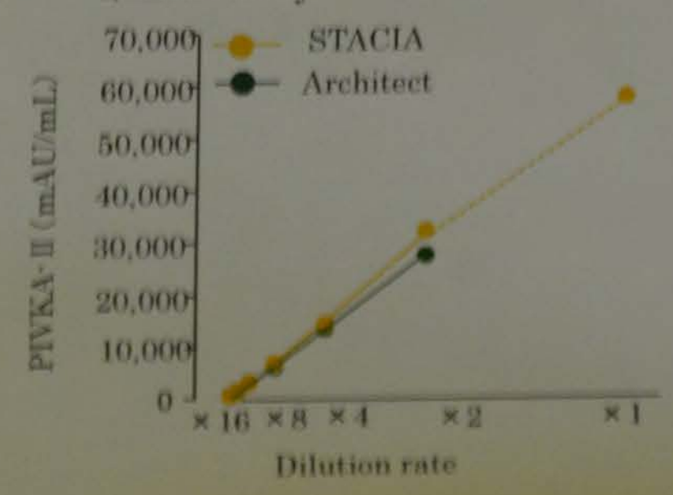
Architect	Control			pool serum	
	L	M	H	1	2
Mean (mAU/mL)	53.39	507.16	10,469.25	31.69	102.93
SD	1.9	21.1	383.8	1.9	3.3
CV(%)	3.6	4.2	3.7	6.0	3.2

STACIA	Control		pool serum	
	I	II	1	2
Mean (mAU/mL)	71.6	299.9	35.1	122
SD	3.1	15.5	2.7	8.2
CV(%)	4.4	5.2	7.7	6.7

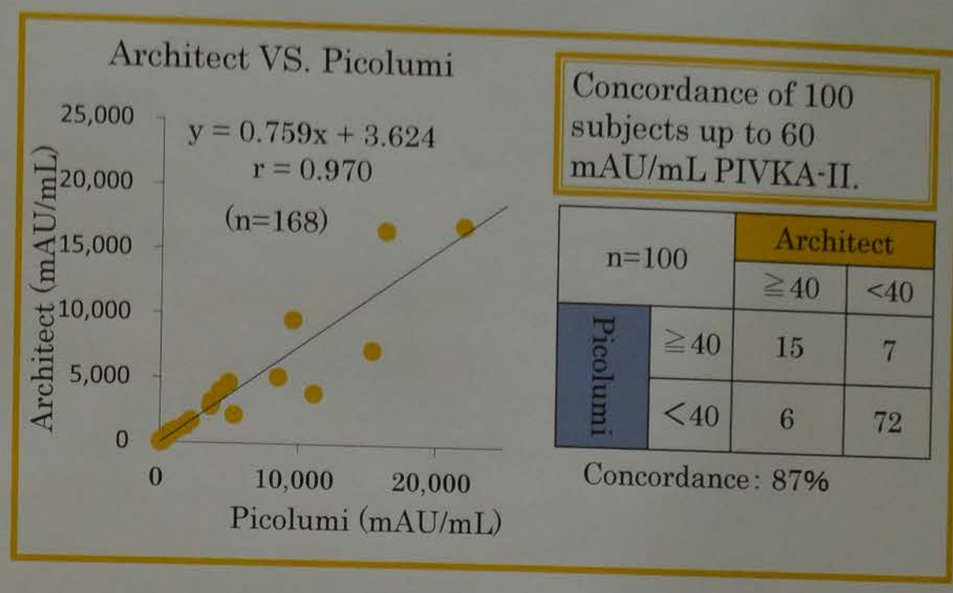
3) Limit of Detection



4) Linearity



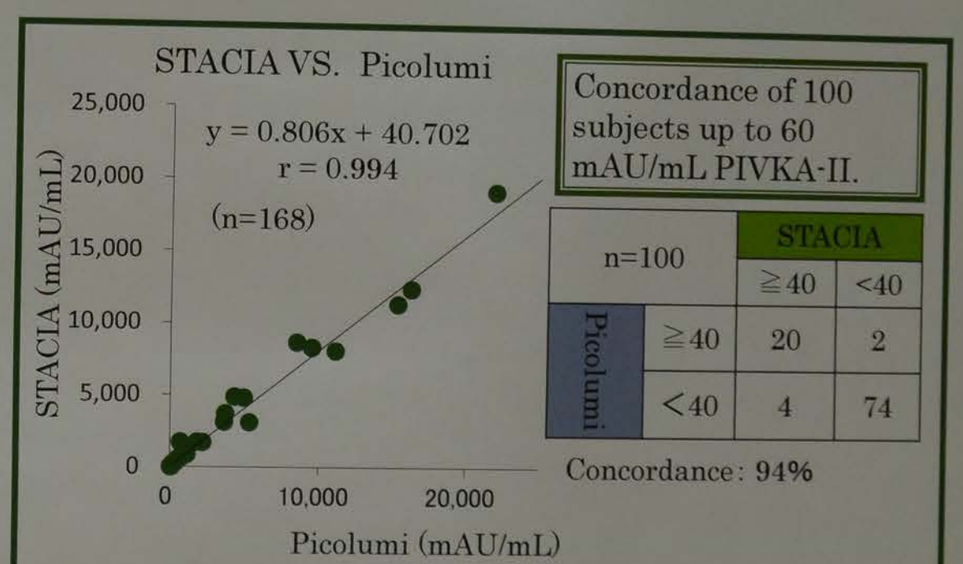
5) Correlation and Concordance Around Cut-off



Concordance of 100 subjects up to 60 mAU/mL PIVKA-II.

n=100		Architect	
		≧40	<40
Picolumi	≧40	15	7
	<40	6	72

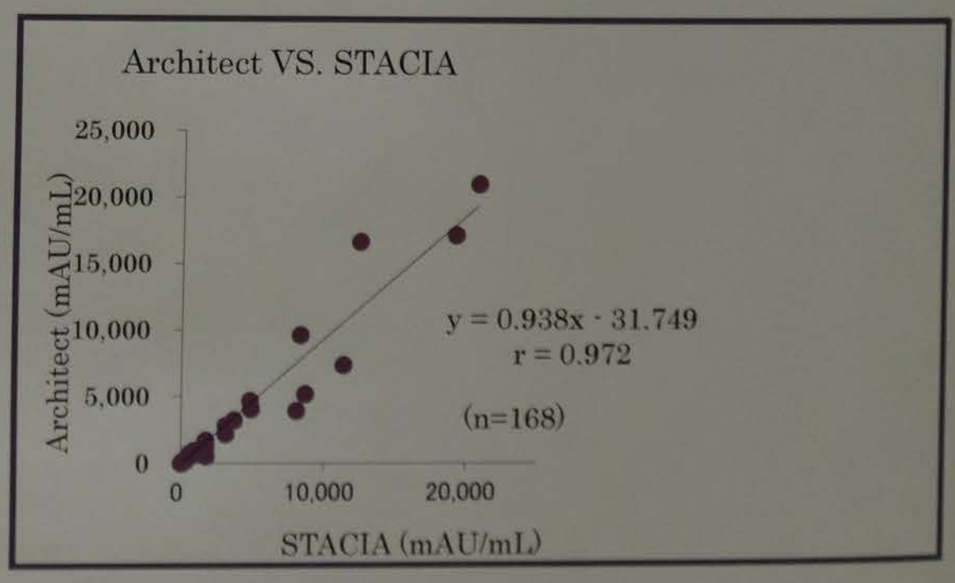
Concordance: 87%



Concordance of 100 subjects up to 60 mAU/mL PIVKA-II.

n=100		STACIA	
		≧40	<40
Picolumi	≧40	20	2
	<40	4	74

Concordance: 94%



3. Summary

① Within-run reproducibility, day-to-day reproducibility and limit of detection were satisfactory with the two assays.

② Although the highest calibrator for Architect is 30,000 mAU/mL and 75,000 mAU/mL for STACIA, we confirmed the linearity of the both assays to be satisfactory up to 30,000 mAU/mL.

③ Correlation of Architect or STACIA against Picolumi was satisfactory while concordance of STACIA and Picolumi around the cut-off (40 mAU/mL) was better.

④ By the correlation slopes, PIVKA-II of Architect was the lowest, that of STACIA was in the middle, and that of Picolumi the highest.

Immunology

PB-29

The comparison of highly sensitive HBsAg assay (HBsAg-HQ) with HBVDNA assay

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Introduction

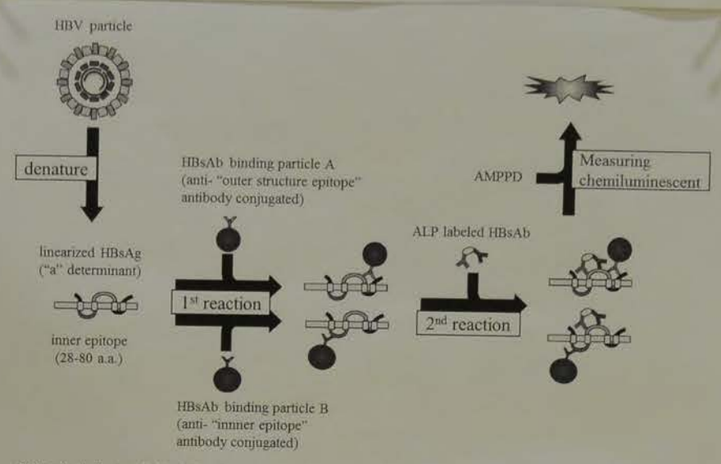
- HBVDNA assay has used as the standard method for detecting low viral loads, such as HBV carriers and immunosuppressive therapy.
- But, the assay was too expensive and time-consuming.
- Recently, a highly sensitive chemiluminescent enzyme immunoassay for HBsAg (HBsAg-HQ®) was developed. HBsAg-HQ is simple, less expensive, and expected to be a surrogate marker of HBVDNA.
- In this study, we examined the relationship between HBsAg-HQ and HBVDNA assay (table 1).

Table 1

HBsAg-HQ	vs	HBVDNA
chemiluminescent enzyme immunoassay	Principle	Taqman PCR
Hepatitis B surface antigen	Measuring object	Hepatitis B DNA
Low	Cost	High
Short (about 1 hour)	time	Long (about 1 day)
Full auto	Operator controls	Semi auto

Materials and methods

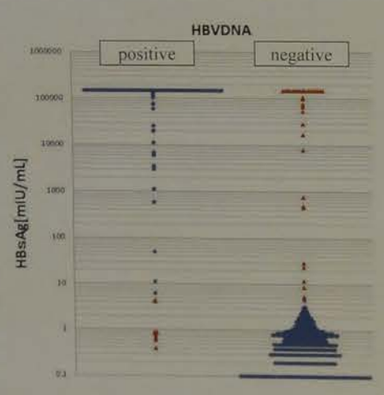
- Materials
  495 serum samples were collected from June 2014 to July 2014.
- Methods
  - HBsAg-HQ: the two-step sandwich assay based on a chemiluminescent enzyme immunoassay (Figure 1).
  - HBVDNA assay: TaqMan PCR assay.
- Strategies
  - In this study, no detectable HBVDNA and HBsAg level <5.0 mIU/mL, respectively defined as negative result.
  - We examined concordance rate of HBsAg-HQ and HBVDNA assay.
  - Moreover, we studied mismatch cases individually.



Shinkai, N. Application of a Newly Developed High-Sensitivity HBsAg Chemiluminescent Enzyme Immunoassay for Hepatitis B Patients with HBsAg Seroclearance. Journal of Clinical Microbiology, 51(11), 3484-3491, 2013

Figure 1 Measurement principle of HBsAg-HQ

Result 1 : concordance rate



▲:Miss match

2-way contingency table

		HBVDNA assay	
		Positive	Negative
HBsAg-HQ	Positive	81	35
	Negative	9	370

concordance rate: **91.1%**

Result 2 : miss match case

Background of miss match case	The number of case	HBsAg-HQ	HBVDNA
administrating nucleoside/nucleotide analogues(NAs)	28	+	-
non-treated HBV carriers	5	+	-
	2	-	+
during immunosuppressive therapy	4	-	+
HBV reactivation cases	2	-	+
treated with antimetabolite	1	+	-
treated with antimetabolite	1	+	-
treated with antiviral drug for HIV infection	1	-	+

Discussion

- The results of the comparison between HBsAg-HQ and HBVDNA have High concordance rate (91.1%). However, there were miss match cases.
- We thought that there are advantage and disadvantage by cases.
- For example…

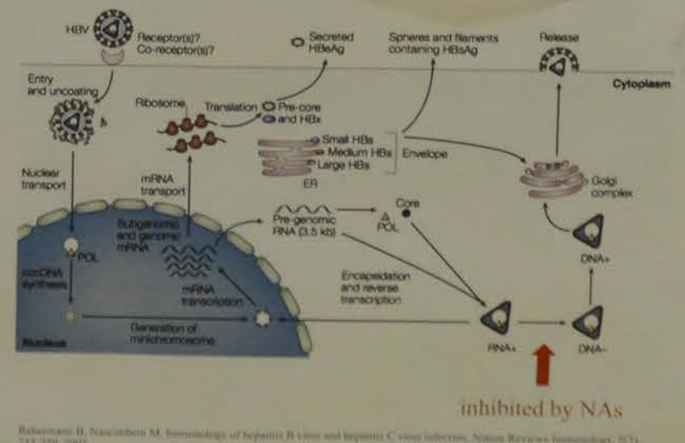
NAs *(HBsAg-HQ+, HBVDNA-)*
HBVDNA is negative because DNA synthesis is inhibited by NAs (Figure 2).

Non-treated HBV carriers *(HBsAg-HQ+, HBVDNA- or HBsAg-HQ-, HBVDNA+)*
The natural history of hepatitis B is variable, complex and still not completely well defined. We thought that there were the various pattern of HBsAg and HBVDNA*.

Immunosuppressive therapy *(HBsAg-HQ-, HBVDNA+)*
2 cases of the results in 4 cases were probably the accidental positive because there were no positive cases before and after. 1 of 4 cases were the continuously low positive cases (no HBV reactivation). Last 1 case developed HBV reactivation after one year.

HBV reactivation*(HBsAg-HQ-, HBVDNA+)*
HBsAg-HQ results were negative because HBVDNA assays are more high sensitive methods than HBsAg-HQ.

*Jay H Hoofnagle, Management of Hepatitis B: Summary of a Clinical Research Workshop. HEPATOLOGY, 45(4), 1056-1075, 2007



inhibited by NAs

Beberniann B, Nassimbeni M. Immunology of hepatitis B virus and hepatitis C virus infection. Nature Reviews Immunology, 3(3), 235-250, 2003

Figure 2 HBV life cycle

Conclution

Although 8.9% of cases showed mismatch results, HBsAg-HQ was high concordance rate, and thought to be substituted for HBVDNA assay.

QR code of Department of Clinical Laboratory, Japanese Red Cross Kyoto Daiiti Hospital

Immunology

PB-30

Significance of rapid assessment of aldosterone-renin ratio in establishing a diagnosis of primary aldosteronism

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[1]Clinical Laboratory , Department of Medical Technology, Ise Red Cross Hospital
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Introduction

- Primary aldosteronism (PA) is known as a typical disease for secondary hypertension. It accounts for 5-10 % of hypertensive patients. Therefore PA screening by Aldosterone Renin Ratio (ARR) is recommended for hypertensive patients.
- ARR is calculated by simultaneous measurement of plasma aldosterone concentration (PAC) and active renin concentration (ARC) or plasma renin activity (PRA).
- PA diagnosis by commercial labs requires 3-5 days due to radioimmunoassay (RIA). Rapid PAC and ARC assays that can be done in approximately 10 minutes have been recently developed on a fully automated immunoassay system, Accuraseed. We have evaluated the Accuraseed Aldosterone and Accuraseed renin(ARC) (Wako Pure Chemical Industries, Ltd.) in this study.

Case of outlier samples

Case : A 54-year-old female
Past medical history: Rheumatoid arthritis
Current medical history: The patient was diagnosed with hypertension 3 years ago.PA was suspected by a general practitioner.

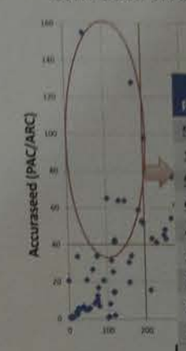
Mesurements of Accuraseed

	Screening	Captopril-challenge test			Saline- infusion test	
	Resting state	0 min	60 min	90 min	before	after
ARC(pg/mL)	3.9	0.8	1.0	0.9	1.5	0.5
PAC(pg/mL)	522.6	519.3	492.0	455.3	611.4	463.8
ARR	134	649.1	492.0	505.9	407.6	927.6
		two months later			3 weeks later	

Image analysis

- CT scan : n.p.
- US:n.p.

Correlation of ARR between the results of Accuraseed and RIA.



RIA PAC/PRA	RIA ARR	Accuraseed PAC/ARC	Accuraseed ARR	Disease
50.4/1.0	50.4	31.0/0.2	155.0	Bilateral adrenal tumor
122.0/0.7	174.3	459.9/3.6	127.8	PA(definition by AVS)
51.6/0.5	103.2	137/2.1	65.2	Electrolyte abnormality
93.1/0.7	133.0	127.9/2.0	64.0	PA(definition by AVS)
44.6/0.3	148.7	89.2/1.4	63.7	PA suspected case
36.9/0.2	184.5	64.8/1.1	58.9	PA suspected case
97.9/0.5	195.8	156.0/3.0	52.0	PA(definition by AVS)
96.3/0.5	192.6	166.8/3.2	52.8	PA(definition by AVS)
84.7/0.7	121.0	107.7/2.6	41.4	PA suspected case
72.8/0.6	21.3	102.4/2.4	42.7	Cushing's syndrome
91.7/0.5	183.0	142.4/1.1	129.5	PA(definition by AVS)

Flow of diagnosis at Ise Red Cross Hospital

Hypertensive patients
↓
Simultaneously measured PAC and ARC
↓
PAC/ARC ratio over 40
↓
Captopril-challenge test
PAC/ARC ratio over 40
↓
Furosemide-upright test / Saline-infusion test / ACTH stimulation test
↓
adrenal venous sampling (AVS)

Case of outlier

Adrenal venous PAC after ACTH loading
Accuraseed : 14,000pg/mL
RIA : 7,430pg/mL

Before	RIA Cortisol (ug/dL)	RIA PAC (pg/mL)
Left adrenal vein:distal	167.0	2767.8
Right adrenal vein	88.4	3364.5
IVC	8.4	97.5

After	RIA Cortisol (ug/dL)	RIA PAC (pg/mL)	RIA PRA (ng/mL/hr)	RIA ARR	Accuraseed Cortisol (ug/dL)	Accuraseed PAC (pg/mL)	Accuraseed ARC (pg/mL)	Accuraseed ARR
Left adrenal vein :distal	1170.0	34000.0	-	-	1010.0	30730.0	2.8	-
Right adrenal vein	1080.0	13000.0	-	-	944.0	14456.0	2.9	-
Left adrenal vein :proximal	910.0	7430.8	-	-	860.0	17220.0	2.6	-

	PAC/Cortisol ratio RIA	PAC/Cortisol ratio Accuraseed	Ratio(left/right) RIA	Ratio(left/right) Accuraseed
Left adrenal vein:distal	29.1	30.4	2.4	2.0
Right adrenal vein	12.0	15.3	-	-
Left adrenal vein:proximal	8.2	20.0	0.7	1.3

RIA 2.4
Accuraseed 2.0
→ Aldosteron-producing adenoma

Diagnostic criterion for PA ①absolute level of PAC after ACTH loading ≧14,000pg/mL
②≧3.0 of A/A-R
③ ≧3.0 PAC of LA and ≦1.0 of CL-R

The patient was diagnosed with suspicious Aldosteron-producing adenoma → Spironolactone treatment

Captopril challenge test positive patients : 17

→ PA suspected patient : 11

CT scan → ・no adrenal mass : 6
・adrenal mass : 5

Case of AVS → PA diagnosed after AVS :5

No.	Accuraseed ARC	Accuraseed PAC	Accuraseed ARR (PAC/ARC)	RIA PRA	RIA PAC	RIA ARR (PAC/PRA)	Confirmatory testing	Image analysis	Result of AVS	Therapy
1	2.0	127.9	64.0	0.7	93.1	133.0	Captopril/ACTH	Left adrenal mass	BHA	medicine
2	2.3	142.7	62.0	0.4	113.0	282.5	Captopril/saline	Right adrenal mass	BHA	medicine
3	0.5	506.4	1012.8	0.1	436.0	4360.0	Captopril/saline	Right adrenal mass	S-APA	OP
4	1.1	142.4	129.5	0.5	91.7	183.0	Captopril/saline	No mass	S-APA	OP
5	3.6	459.9	127.8	0.7	122.0	174.3	Captopril/saline	No mass	BHA	medicine

Based on the PA diagnosis, drug treatment was changed to three drugs in case 1, 2, and to two drugs in case 5.

【Materials and Method】

Subject : 122 patients suspected of having secondary hypertension (From January 2016 to July 2016).

Method : Comparison of PAC, PRA, ARC and ARR in PA diagnosis

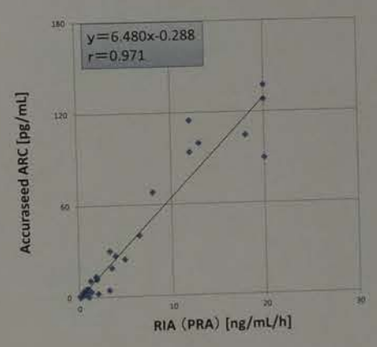
Instrument and Reagent

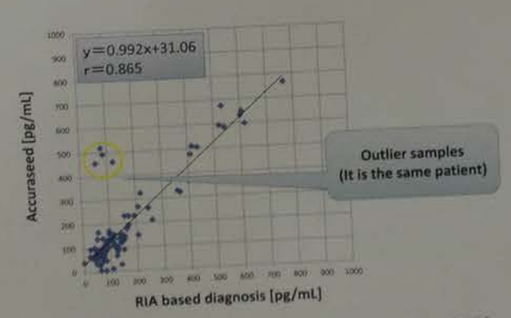
Instrument : Accuraseed (Wako Pure Chemical Industries, Ltd.)

Reagent : Accuraseed Aldosterone and Accuraseed renin RIA kit (SPAC-S aldosterone kit and renin activity (PRA) FR kit; Fujirebio Inc)

Method : The PAC and ARC assays were measured by using the Accuraseed and the RIA assays.

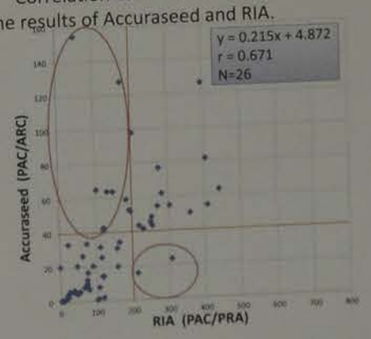
Correlation between the results of Accuraseed ARC and RIA PRA.





Correlation of PAC between the results of Accuraseed and RIA in 122 patients with possible secondary hypertension

Correlation of ARR between the results of Accuraseed and RIA.



11 discrepancy cases were observed in the correlation study between Accuraseed and RIA results.

Results

- In terms of PAC and ARC, Accuraseed results showed good correlation with RIA assay results (PAC:y=0.992x+31.06 , r=0.865 , ARC v.s. PRA:y=6.480x -0.288 r=0.971).
- In comparison between Accuraseed and RIA based diagnosis, there were 8 discrepancy cases and 2 of them took Captopril challenge test.
- Captopril challenge testing was performed in 25 cases with resultant 8 positives.
- CT scanning of the 8 positives revealed no adrenal masses, but 2 of them were diagnosed with PA after adrenal venous sampling (AVS).

Correlation of ARR between the results of Accuraseed and RIA.

RIA PAC/PRA	RIA ARR	Accuraseed PAC/ARC	Accuraseed ARR	Disease
64.0/0.3	213.3	36.0/2.3	15.7	Electrolyte abnormality
123.0/0.4	307.5	65.5/2.8	23.4	Essential hypertension

Discussion 1

- Early diagnosis of PA is important to avoid the risk of cardiovascular disease cased by excessive secretion of aldosterone.
- PA is a curable disease with adequate medical treatment. Therefore rapid screening is key to diagnose PA in refractory hypertension. After installing Accuraseed into our hospital, the number of ARR and Captroil-challenge test could increase (1.6-1.7 times). Consequently, we can diagnose 3 or more PA patients.
- ARR with AVS is important screening test for PA because CT scanning can't detect microadenoma smaller than 6mm. In addition, ARC level is also essential because ARR is calculated from ARC at a factor of denominater. Accuraseed renin (ARC) and Accuraseed aldosterone can provide ARR value rapidly with high sensitivity.

Discussion 2

- We observed 11 cases of discrepancy in this ARR study. 4 of them were determined as PA in the confirmed diagnosis. It indicates the significance of high sensitive ARC diagnosis. On top of that, the significance of rapid diagnosis in confirmatory testing and AVS.
- We can use a selective aldosterone blocker as a first-line drug for the patients who are suspected as PA in our confirmatory test. This means that we can prescribe medicine corresponding to a patient condition based on scientific evidence.

Conclusion

- Simultaneous measurement of ARC and PAC in routine diagnostic test allows us to diagnose PA at an early stage.
- Accuraseed is useful for rapid PA diagnosis in outpatient care. It gives a lot of benefits to the patients like reducing RTC and changing antihypertensive medication.

PB-31

Evaluation of free light chain assay in serum

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Takatsugu Honda,Nahoko Sumi,Mari Morimoto,Tatsuya Nishimiya
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INTRODUCTION

Monoclonal immunoglobulin free light chain (FLC) are important tumor markers often present in serum of patients with plasmaproliferative disease disorders. Serum FLC were measured with two commercial reagent kits. Objectives were to evaluate monoclonal antibody-based nephelometric N Latex FLC(Siemens). We assessed the analytical performance of the N Latex FLC assays and compared it with the Freelight™(Binding Site) assays.

METHODS

- Analytical precision was assessed using control , linearity on FLC control and samples from monoclonal elevations of FLC. We demonstrated lot-to-lot consistency with variation of plural N Latex FLC reagent and calibrator.
- Serum samples submitted for routine FLC analysis (31 samples) were analyzed by both methods (N Latex FLC and Freelight™) according to the manufacturers' instructions, and in addition performed by immunofixation(IF).
- A useful clinical case of FLC analysis

	N Latex FLC (Siemens)	Freelite™ (Binding Site)
Principle	nephelometry	nephelometry
Antibody	monoclonal	polyclonal
Reference value		
Kappa	6.7-22.4 mg/L	3.3-19.4 mg/L
Lamda	8.3-27.0 mg/L	5.7-26.3 mg/L
κ/λ ratio	0.31-1.56	0.26-1.65

RESULT

BASIC PERFORMANCE

Precision

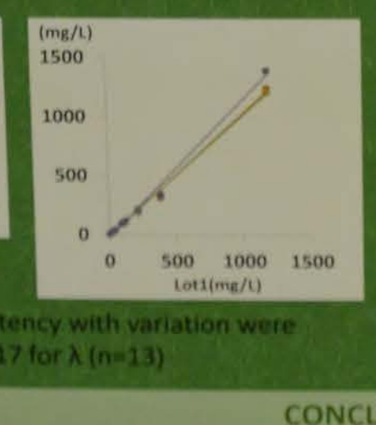
Inter assay(n=10)

	Control 1 CV(%)	Control 2 CV(%)
FLC kappa	3.15	3.29
FLC lamda	3.53	3.58

Reproducibility intra assay (n=7day)

	Control 1 CV(%)	Control 2 CV(%)
FLC kappa	3.49	3.10
FLC lamda	4.26	6.72

Linearity

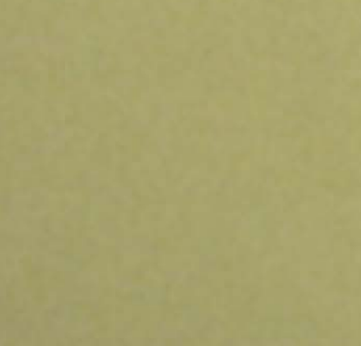


The intra- and inter assay CVs were 3.15-6.72%.
The linearity of assay were excellent.

Lot to Lot : FLC-κ

n=13

	Lot	Reagent Lot	Cal Lot	Regression equation
	Lot1	A	A	
◆	Lot2	A	B	y=0.920x-2.181
■	Lot3	A	C	y=0.964x-1.370
▲	Lot4	B	A	y=0.935x+0.317
×	Lot5	B	B	y=0.914x+0.151
・	Lot6	B	C	y=0.892x+2.190



Lot to Lot : FLC-λ

n=13

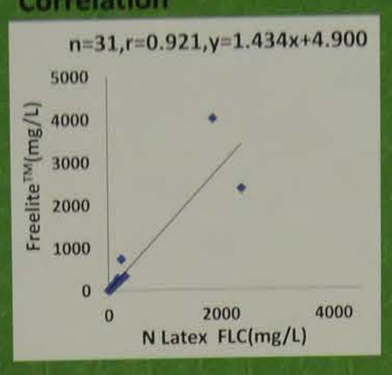
	Lot	Reagent Lot	Cal Lot	Regression equation
	Lot1	A	A	
●	Lot2	A	B	y=1.021x-5.980
●	Lot3	A	C	y=1.041x-12.552
●	Lot4	B	A	y=1.019x-7.462
●	Lot5	B	B	y=1.168x-23.184

The slopes of lot-to-lot consistency with variation were 0.892-0.964 for κ and 1.02-1.17 for λ (n=13)

Comparison of N Latex FLC and Freelite™

FLC-κ

Correlation



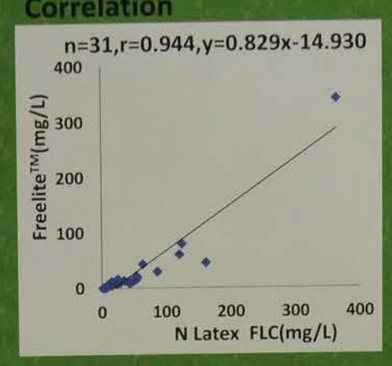
Concordance

N Latex FLC ↓ \ Freelite™ →	<3.3	3.3-19.4	>19.4
<6.7	1	0	0
6.7-22.4	0	9	1
>22.4	0	2	18

Different : 3/31 9.7%
Agreement : 28/31 90.3%
Cohen's Kappa Coefficient:0.802

FLC-λ

Correlation



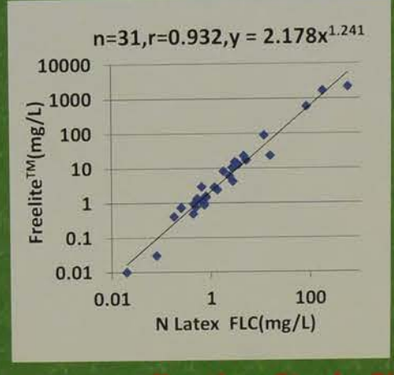
Concordance

N Latex FLC ↓ \ Freelite™ →	<5.7	5.7-26.3	>26.3
<8.3	4	0	0
8.3-27.0	3	9	0
>27.0	0	9	6

Different : 12/31 38.7%
Agreement :19/31 61.3%
Cohen's Kappa Coefficient:0.407

FLC Ratio

Correlation



Concordance

N Latex FLC ↓ \ Freelite™ →	<0.26	0.26-1.65	>1.65
<0.31	2	2	0
0.31-1.56	0	10	3
>1.56	0	0	14

Different : 5/31 16.1%
Agreement : 26/31 83.9%
Cohen's Kappa Coefficient:0.722

Immunofixation Study:FLC ratio

N Latex FLC

	κ/λ ratio positive	κ/λ ratio negative	total N
IF positive	16	5	21
IF negative	2	8	10
total N	18	13	31

Agreement : 24/31 77.4%
Cohen's Kappa Coefficient:0.520

Freelite™

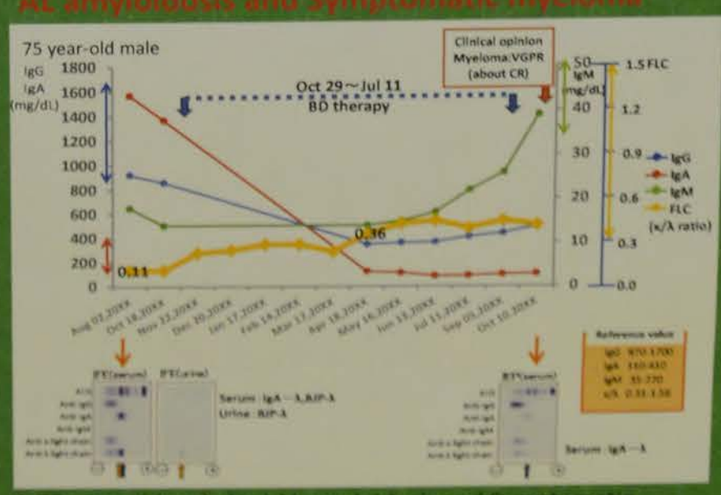
	κ/λ ratio positive	κ/λ ratio negative	total N
IF positive	15	6	21
IF negative	4	6	10
total N	19	12	31

Agreement : 21/31 67.7%
Cohen's Kappa Coefficient:0.299

For method comparison, the slopes of regression line were 1.434(FLC-κ) ,0.829(FLC-λ) and 2.178(FLC-κ/λ ratio), furthermore the between method variances were 9.7%(FLC-κ) ,38.7%(FLC-λ) and 16.1%(FLC-κ/λ ratio) when comparing with the Freelite™.
Good agreement was observed for the κ/λ ratio (83.9%). However, N Latex FLC assay was higher agreement rate of κ/λ ratio against IF than Freelite™(77.4% vs 67.7%).

CASE REPORT

AL amyloidosis and Symptomatic myeloma



A 75-year-old male had AL amyloidosis and Symptomatic myeloma(IgA-λ ,BJP-λ).His FLC ratio changed before or after BD therapy from 0.11 to 0.36.His FLC ratio is normal ever since and has a complete remission. This report was a useful clinical case of FLC analysis.

CONCLUSIONS

The N Latex FLC assay has good precision.We have good data performances and correlation in comparison with two FLC method.However,because Freelight ™ was higher than N Latex in FLC-κ and lower in FLC-λ,it should not be used to mix the respective value in clinical.

Immunology

PB-32

PB-32

Novel Enzyme Immunoassay System for Simultaneous Detection of Seven Subclasses of Anti-Phospholipid Antibodies For Differential Diagnosis of Anti-Phospholipid Syndrome

Kazusa HARA[1], Yukari MOTOKI[1], Toshiyuki SAKATA[2], Junzo NOJIMA[1]

1. Department of Laboratory Science, Faculty of Health Science Yamaguchi University Graduate School of Medicine, Japan
2. I.L. Japan Co.,Ltd.

Background

Anti-phospholipid antibodies (aPLs) are a heterogeneous group of autoantibodies that can be detected by Enzyme-Linked Immunosorbent Assay (ELISA) or the phospholipid-dependent coagulation assays. These antibodies are frequently found in the plasma of patients with a variety of autoimmune diseases, particularly systemic lupus erythematosus(SLE). The presence of aPLs is associated with clinical events such as arterial and/or venous thrombosis and recurrent fetal loss. It is now generally accepted that aPLs do not bind directly to the negatively charged phospholipid itself but rather to complexes of the phospholipid and phospholipid-binding proteins and that the most common and well-characterized antigenic targets are β_2GPI. Several aPL-ELISAs such as aCL-ELISA and aβ_2GPI-ELISA were established for the diagnosis of APS. However, the clinical importance of the immunoglobulin subclasses of aCL and/or aβ_2GPI has not yet been clearly determined. Therefore, we developed an up-to-date enzyme immunoassay (EIA) system using the AcuStar® automated analyzer for parallel detection of seven subclasses of aPLs: aCL and aβ_2GPI, each of IgG, IgM, and IgA classes, plus newly introduced anti-β_2GPI-domain 1 antibodies (aDomain1) of IgG class. We applied this automated simultaneous assay system to the highly specific detection of these aPL subclasses in 276 normal healthy volunteers and 138 patients with SLE, including those with arterial or venous thrombosis and without thrombosis.

Method

aPLs-EIA

We established an up-to-date enzyme immunoassay (EIA) system using the AcuStar® automated analyzer for parallel detection of seven subclasses of aPLs: anti-cardiolipin antibodies (aCL) and anti-β_2-glycoprotein I antibodies (aβ_2GPI), each of IgG, IgM, and IgA classes, plus newly introduced anti-β_2-glycoprotein I-domain 1 antibodies (aDomain1) of IgG class. This assay uses chemiluminescence. As an example, aPLs in patient serum bind to magnetic micro particles coated with complex of bovine cardiolipin and human β_2GPI, and combined with isoluminol-labeled anti human IgG monoclonal antibody or anti human IgM monoclonal antibody. Then, luminescence intensity is measured by luminometer. (Fig. 1)

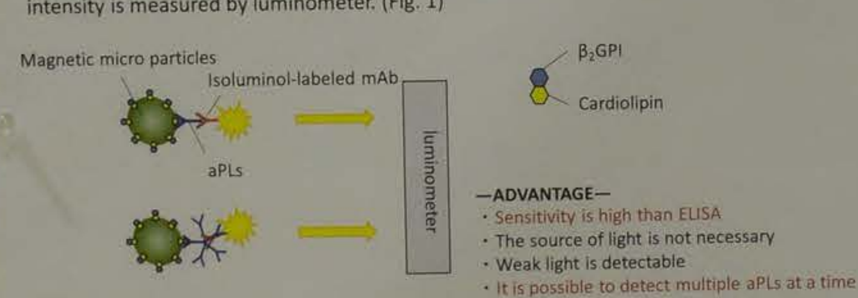


Figure 1 . Measurement principle of aPLs-EIA (aCL)

Usefulness analysis of aPLs-EIA system

We measured each aPLs concentration in 276 normal healthy volunteers (189 females, 87 males; 20-61 years of age; mean, 37 years) and 138 patients with SLE (129 females, 9 males; aged 8-82 years, mean 43 years): involved 29 patients with arterial thrombosis, 16 patients with venous thrombosis, and 93 patients without any thromboembolic complications. We examined the correlation of measurement value about seven subclasses of aPLs, and analyzed association between history of thrombosis and each aPLs concentrations statistically. To examine usefulness aPLs-EIA system, we compared this system with aCL/β_2GPI ELISA kit which is commonly used in Japan to diagnose APS. All statistical analyses were performed by use of StatFlex statistical software (Ver. 6.0, Arthch Inc., Japan)

Result・Discussion

Cut-off value and Correlation between concretions of any two aPLs in the patients with SLE

The concentrations of IgG-aCL, IgM-aCL, IgA-aCL, IgG-aβ_2GPI, IgM-aβ_2GPI, IgA-aβ_2GP, and IgG-aDomain1 measured in the 276 normal healthy control subjects and 138 patients with SLE were logarithmically transformed to adjust for skewness of the distribution before performing the statistical analyses. The upper 98 percentile point of each antibody concentration in the 276 normal controls was set as the cut-off value. The cut-off value for the seven antibody concentrations and positive number of 138 patients with SLE were as follows "Table 1". In the plasma of the 138 patients with SLE, a highly significant correlation was found between the concentrations of IgG-aCL and IgG-aβ_2GPI (r = 0.86; Fig. 2A), IgM-aCL and IgM-aβ_2GPI (r = 0.80; Fig. 2B), IgA-aCL and IgA-aβ2GPI (r = 0.95; Fig. 2C), IgG-aDomain1 and IgG-aCL (r = 0.80; Fig. 2D), and IgG-aβ_2GPI-Domain1 and IgG-aβ_2GPI (r = 0.84; Fig. 2E).

Table 1. Cut-off value and positive number with normal healthy controls

	Cut-off value	Positive number
aCL-IgG	< 20.1 U/mL	53 (38.4%)
aCL-IgM	< 20.2 CU	6 (4.3%)
aCL-IgA	< 3.7 CU	59 (42.8%)
aβ_2GPI-IgG	< 18.5 CU	56 (40.6%)
aβ_2GPI-IgM	< 8.9 CU	18 (13.0%)
aβ_2GPI-IgA	< 6.0 CU	51 (37.0%)
aDomain1-IgG	< 6.3 CU	52 (37.7%)

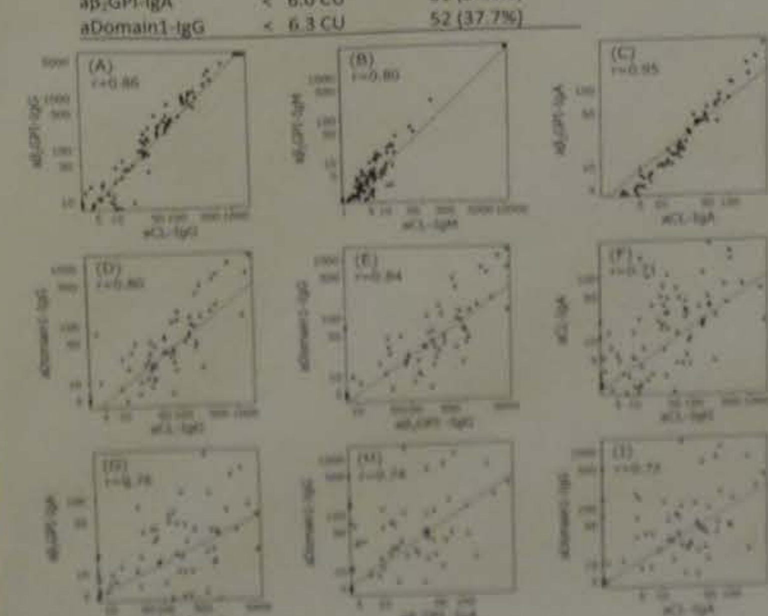


Figure 2. Correlation between concretions of any two aPLs in the patients with SLE

Result・Discussion

Relation between the each aPL concentration and the prevalence of arterial and/or venous thrombosis.

The patients with SLE were divided into three groups according to their known episodes of complications: arterial thrombosis (n = 29), venous thrombosis (n = 16), and no thrombosis (n = 93). As a result, the median IgG-aCL (Fig. 3A), IgA-aCL (Fig. 3C), IgG-aβ_2GPI (Fig. 3D), and IgA-aβ_2GPI (Fig. 3F), IgG-aDomain1 (Fig. 3G) concentrations were significantly higher in the SLE patients with arterial and/or venous thrombosis than in those SLE patients without thrombosis. The results of multivariate logistic regression analysis of factors associated with thrombosis are shown in Table 2. The most significant risk factor associated with arterial thrombosis was the presence of IgG-aDomain1 (aOR = 8.28, 95% CI = 3.20–21.4, Table 2). In contrast, the risk factor most closely associated with venous thrombosis was the presence of IgG-aβ_2GPI (aOR = 17.1, 95% CI = 1.03–285, Table 2). In this study, the type of antibodies associated with pathogenesis of arterial and venous thrombosis were suggested that may be different.

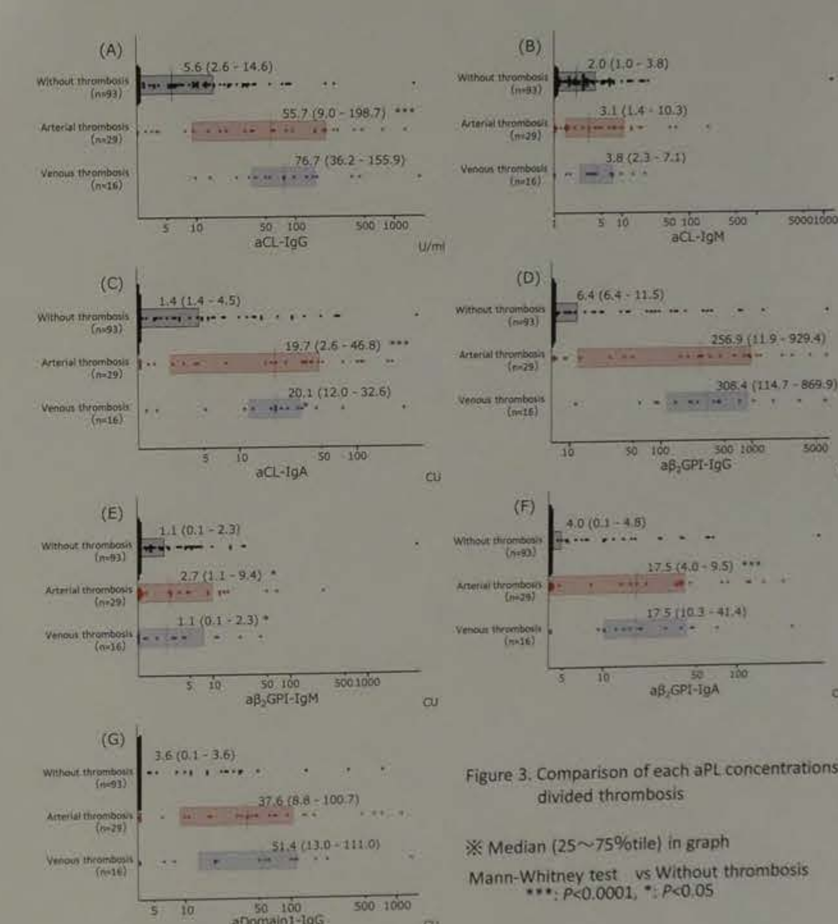


Figure 3. Comparison of each aPL concentrations divided thrombosis

※ Median (25~75%tile) in graph

Mann-Whitney test vs Without thrombosis
***: $P<0.0001$, *: $P<0.05$

Table 2. Risk factor of arterial and/or venous thrombosis with SLE

	Arterial thrombosis OR (95% CI)	Venous thrombosis OR (95% CI)
aCL-IgG	0.65 (0.12–3.43)	2.23 (0.27–18.4)
aCL-IgM	1.67 (0.22–12.9)	0.71 (0.07–7.17)
aCL-IgA	0.62 (0.07–5.55)	3.98 (0.67–23.8)
aβ_2GPI-IgG	1.54 (0.20–11.9)	**17.1 (1.03–285)**
aβ_2GPI-IgM	1.18 (0.30–5.00)	0.68 (0.17–2.67)
aβ_2GPI-IgA	1.08 (0.17–6.93)	4.69 (0.79–20.0)
aDomain1-IgG	**8.28 (3.20–21.4)**	0.87 (0.15–5.08)

Comparison clinical usefulness of each assays , about thrombosis and LA activity

The measurement results of the plasma of the 138 patients with SLE by this new EIA system were compared with those by the most commonly used standard ELISA kit for aCL/β_2GPI in Japan. ROC analysis showed that the accuracy of predicting thrombotic complications based on the test results of aCL/β_2GPI-ELISA was 0.80 in terms of the AUC. In contrast, the combined test results of this EIA system raised the accuracy (AUC) of predicting thrombotic complications to 0.88 (Fig. 4). Besides, ROC analysis revealed that the accuracy (AUC) of predicting positive LA activity based on the test results of aCL/β_2GPI-ELISA alone was 0.88. Furthermore, by combining the test results of IgG-aDomain1 and IgG-aβ_2GPI using the regression model, the accuracy of predicting the presence of LA activity expressed as the AUC rose to 0.93 (Fig. 5). As for thrombotic complications and LA activity, it was suggested that aPL-EIA was higher diagnostic usefulness than aCL/β_2GPI-ELISA.

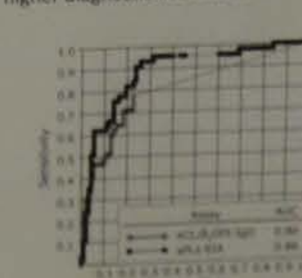


Figure 4. ROC with or without thrombosis

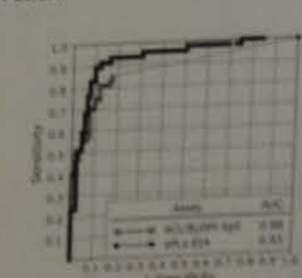
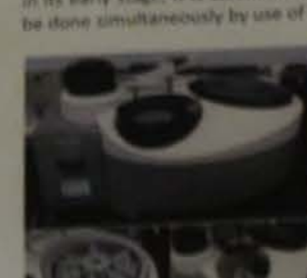


Figure 5. ROC with or without LA activity

Finally

In this study, patients with SLE frequently possess various subclasses of aPLs such as IgG-aCL, IgA-aCL, IgG-aβ_2GPI, IgA-aβ_2GPI, and IgG-aDomain1. The occurrence of recurrent thrombotic and obstetric complications in SLE apparently depends on variable combinations of these subclasses of aPLs. Therefore, in the differential diagnosis of APS in its early stage, it is essential to detect the full spectrum of aPL subclasses, which can be done simultaneously by use of this new, automated EIA system.



The 32nd World Congress of Biomedical Laboratory Science COI Disclosure Information

Yamaguchi Univ. Kazusa HARA

I have no financial relationships to disclose.

Immunology

PB-33

PB-33

Evaluation of the basic performance of BS-NIA IgG4

Yoko Usami, Nau Ishimine, Kazuhiro Nagata, Kenji Kawasaki,
Mitsutoshi Sugano, Takayuki Honda
Department of Laboratory medicine, Shinshu University Hospital, Nagano, Japan

Background

IgG4-related disease (IgG4-RD) is a newly recognized disorder characterized by elevated serum IgG4 concentration and increased IgG4-positive plasma cell infiltration in tissue. Since comprehensive diagnostic criteria for IgG4-RD were proposed in 2011, the demand for IgG4 level detection has increased. In this study, we evaluated the basic performance of BS-NIA IgG4, which is widely used in Japan and also used in the study for determining cutoff value of IgG4-RD diagnostic criteria.

Materials and Methods

IgG4 level was measured with a Siemens BN II nephelometer using BS-NIA IgG4 (The Binding site). Precision was validated by ten replicate measurements for intra-day measurements and once a day for 10 days for inter-day measurements, using pooled serum. In addition, linearity was determined by preparing serial dilutions of serum with IgG4 concentration above the initial measuring range (0.13 -4.41 g/L) and measuring each in triplicate. When IgG4 concentration is higher than 4.14 g/L, sample is automatically diluted and re-measured. The interferences of conjugated and free bilirubin, hemoglobin, chyle, and rheumatoid factor (RF) on IgG4 measurement were also evaluated using Interference Check A Plus and Interference RF (Sysmex).

Results

1, Precisions

Intra-day precision

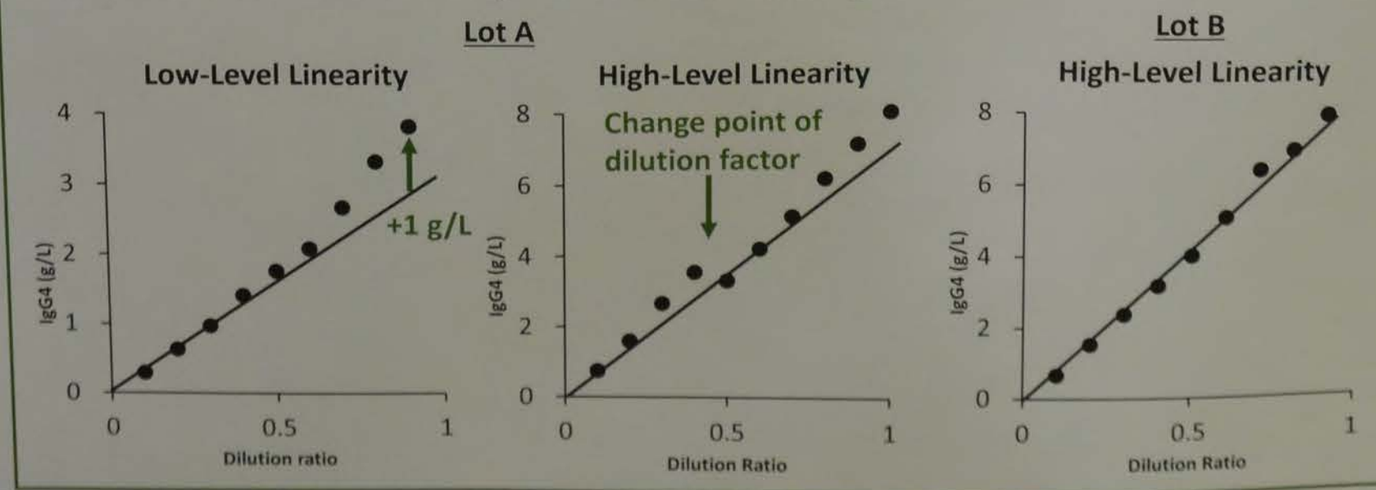
	Mean(g/L)	SD(g/L)	CV(%)
serum①	1.37	0.042	3.03
serum②	2.86	0.078	2.72

Inter-day precision

	Mean (g/L)	SD (g/L)	CV (%)
serum①	1.43	0.055	4.17
serum②	3.16	0.097	3.25

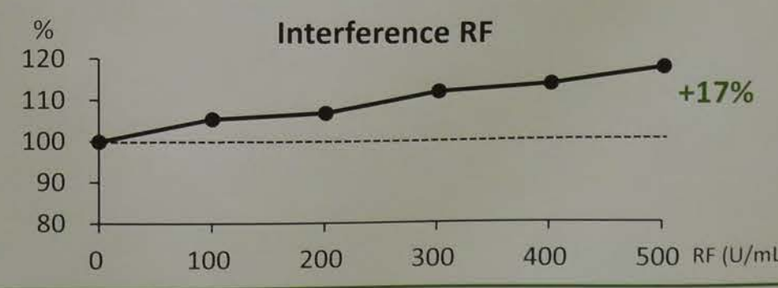
2, Linearity

Although a good linearity was observed from 0.3 to 2 g/L, IgG4 was higher than the expected value at the concentration from 2 to 4 g/L. There was large discrepancy between initial and further dilution measurement. However this discordance was not observed in measurements using two other lots of BS-NIA IgG4.



3, Interferece

The presence of conjugated and free bilirubin, hemoglobin, and chyle did not influence the measurement. Meanwhile, a 17% increase in IgG4 was observed at RF concentration of 500 IU/mL.



Conclusion

The performance of BS-NIA IgG4 for routine measurements was satisfactory, provided that its linearity checked prior to use.

Immunology

PB-34

Changes of serum M2BPGi levels in chronic hepatitis C patients treated with interferon-based or interferon-free therapy

Hidekazu ISHIDA[1)], Atsushi SUETSUGU[2)], Yuriko KATANO[1)], Rina TAUCHI[1)], Yukari OMORI[1)], Nobuyuki FURUTA[1)], Yohei SHIRAKAMI[3)], Hiroyasu ITO[1),3)], Mitsuru SEISHIMA[1),3)]

1) Division of Clinical Laboratory, Gifu University Hospital, 2) Division of Gastroenterology/Internal Medicine, Gifu University Hospital
3) Department of Informative Clinical Medicine, Gifu University Graduate school of Medicine

GIFU UNIVERSITY

Introduction

Background

It has been reported that eradication of Hepatitis C virus (HCV) improves liver fibrosis in chronic hepatitis C patients. Recently, the treatment of chronic HCV infection has been advancing with the advent of direct-acting antiviral agents (DAAs) instead of interferon (IFN)-based therapy.

Aim

We evaluated here the changes of the M2BPGi levels in chronic hepatitis C patients treated with IFN-based therapy or DAAs therapies.

Methods

We measured three markers for liver fibrosis.

M2BPGi; Mac-2 binding protein glycosylation isomer

HISCL M2BPGi Assay Kit Sysmex Corp.

ELF Score; Enhanced liver fibrosis score

ELF Test Siemens Healthcare Diagnostics Inc.

= 2.278 + 0.851 ln(hyaluronic acid *(ng/mL)*)
+ 0.751 ln(amino-terminal propeptide of type III procollagen *(ng/mL)*)
+ 0.394 ln(tissue inhibitor of metalloproteinase 1 *(ng/mL)*)

ALT; alanine aminotransferase

Reagent; QUICK AUTO NEO ALT-JS Shino-Test Corp.
Instrument; JCA-BM6070 JEOL ltd.

Subjects

Ninety-five chronic hepatitis C patients who underwent treatment at Gifu University Hospital.

Treatment	Pegylated-interferon (**PEG-IFN**), Ribavirin (**RBV**) and Simeprevir (**SMV**)	Asunaprevir (**ASV**) and Daclatasvir (**DSV**)	Sofosbuvir (**SOF**) and Ledipasvir (**LDV**)	Sofosbuvir (**SOF**) and Ribavirin (**RBV**)
Number of patients	**7** *patients*	**30** *patients*	**39** *patients*	**19** *patients*
HCV genotype	1b	1b	1b	2a
Duration of therapy	24 *weeks*	24 *weeks*	12 *weeks*	12 *weeks*

Results

†: $P < 0.05$, ‡: $P < 0.01$
Log-transformed data were analyzed by a two-way ANOVA followed by Tukey's post hoc tests.

Discussion

The liver fibrosis markers in chronic hepatitis C patients with eradication of Hepatitis C virus (HCV) improved. We found that liver fibrosis markers of patients with IFN therapy increased among treatment. This finding is IFN therapy eradicated HCV by activating the immune function.

In contrast, we found that liver fibrosis markers of patients with DAAs therapy decreased among and after treatment. We considerd that DAAs therapy eradicated without hepatitis.

The serum M2BPGi level may be influenced not only by liver fibrosis but also by liver inflammation.

Conclusion

M2BPGi is a non-invasive liver fibrosis marker useful for predicting the efficacy of DAAs therapy for chronic hepatitis C patients.

Immunology

PB-35

The examination of ALP isozyme anomaly case profile in our hospital

Yachiyo Endo[1)], Masanori Seimiya[2)], Mariko Watanabe[1)], Haruna Asano[1)], Toshihiko Yoshida[1)], Yuji Sawabe[1)]

[1)]Division of Laboratory Medicine, Chiba University Hospital
[2)]International University of Health and Welfare

CHIBA UNIVERSITY

Objective

The ALP isozyme profile provides an important clue to the diagnosis of pathological conditions. However, an irregular electrophoretic pattern (anomaly) may be accepted due to immunoglobulin-binding or a change of transient carbohydrate chain structure. We examined ALP anomaly cases in our hospital.

About ALP anomary

The ALP is an enzyme hydrolyzing organic phosphorus acid monoester under the alkalinity. There are isoform and isozyme in ALP, and it is usually migrated for 2-4 compartmentation by agarose gel electrophoresis. By the isozymic analysis of the enzyme, the migration image may show an electrophoretic image unlike a normal movement pattern.This is called anomary. Transient hyperphospha-tasemia (THP), immunoglobulin- binding ALP, lipid-binding ALP, tumor-producing ALP etc... are reported in a cause of anomary.

Methods

Sera were from patients who visited our hospital. Written informed consent was obtained from all participants prior to blood sampling, and the study protocol was approved by the Ethics Committee of Chiba University Graduate School of Medicine. ALP activity was measured with an automated biochemical analyzer equipped with the reagents used in the JSCC-recommended method. ALP isozymes were analyzed using Epalyzer II (Helena Laboratories).

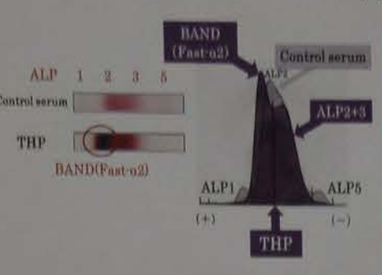
The immunoglobulin-binding ALP cases were identified by countercurrent immunoelectrophoresis or immunofixation.

Results

The cases of ALP anomaly

Between 2007 and 2015, 4,055 patients developed ALP isozyme. A total of 63 cases (1.6%) revealed an anomaly pattern.

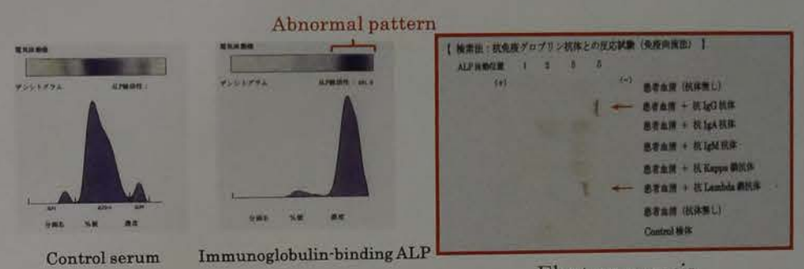
Case1.Transient hyperphosphatasemia(THP)



Real reported case

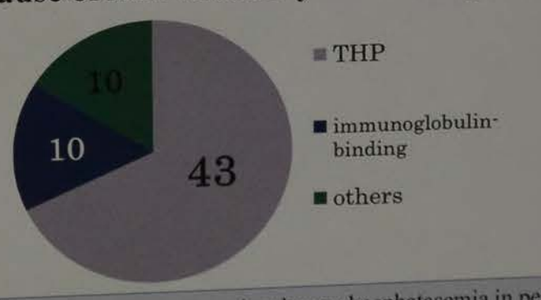
In THP, ALP isozymes exhibit a unique pattern, making it useful for the accurate diagnosis of this condition, such as a band in the fast α-2 region and in the ALP2,3 region. THP mainly occurs in infants and children, ALP activity increases transiently to several thousand unit, but decreases within approximately 2 months, and other laboratory data is normal. Although viral and other infections have been suggested as the cause of this condition, the actual cause currently is unknown.

Case2. Immunoglobulin-binding ALP



With the electrophoretic image, an abnormal pattern is accepted from ALP5 by the cathode side. The ALP isozymic compartmentation is impossible by such a pattern, but is more likely to be caused by combination with immunoglobulin. Therefore, it is necessary to search presence of the combination with immunoglobulin by the electrosyneresis or immunofixation. In this case, the reaction with anti IgG antibody and the anti Lambda chain antibody was detected by electrosyneresis.

Cause of ALP anomary in our hospital

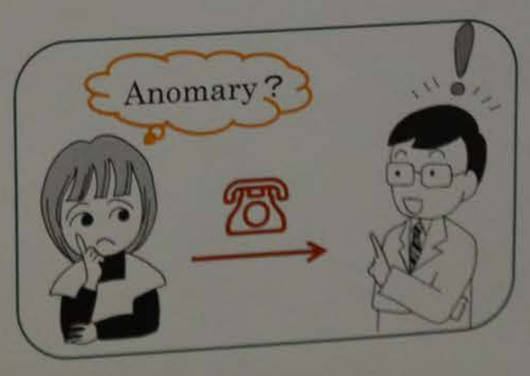


The most common cause was transient hyperphosphatasemia in pediatric patients (THP, 43 cases). ALP electrophoresis revealed a pattern (A band in the fast α-2 region) characteristic of this condition. The other causes of anomaly cases were immunoglobulin-binding ALP (10 cases), and others (10 cases).

Conclusions

A commonly suggested but unproven mechanism for THP or immunoglobulin-binding ALP is impaired clearance of ALP from the circulation. Once patients have been diagnosed with isozyme profile, they do not need to undergo further evaluation, but may be followed-up regularly. It is particularly meaningful for children to avoid undergoing additional tests.

Therefore, the cause of anomaly isozyme profile should be diagnosed. In addition, if the results of ALP isozyme analysis raise a suspicion of this condition, the clinical laboratory scientist should provide the attending physician with information and appropriate comments when reporting test results.



Immunology

PB-36

The examin... case profile in ou...

Mariko Watanabe[1)] Masanori Seimi...
Keiko Saito[1)] Toshihiko Yoshida[1)] ...
1)Division of Laboratory Medicine, Chiba ...
2)International University of Health and ...

[Background]

In the analysis of LD isozyme, abnormal fractions may de detected at unusual positions, which are called anomalies. In our hospital, we have searched for causes of such anomalies wherever possible. In the present study, we analyzed the trend of LD isozyme anomalies found in our hospital.

[Objectives]

To investigate the significance of the implementation of the LD isozyme.

[Materials and Methods]

Serum of patients who visited our hospital and gave informed consent were used. Serum samples were collected from 4,691 patients for whom the measurement of LD isozyme was requested to our clinical laboratory between April 2005 and September 2015.

For the analysis of LD isozyme, Epalyzer and dedicated reagents were used. For anomalies, LD isoenzymes in hemolysates of patients erythrosytes were analyzed to examine gene mutations. For cases without abnormal electrophoretic image with the hemolysates, electrosyneresis or immunofixation (IFE) were conducted.

[Results]

In the past decade, 36 LD isozyme anomalies were detected, which accounted for 0.8% of all tested cases. They included genetic abnormalities (3 cases), enzyme-linked immunoglobulin (14 cases), LD6 (ALDH; 1 cases), and unknown cause (18 cases).

Case 1: H-subunit mutations

Case 3

Only Serum

[Conclus...

Total LD a...
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Immunology

PB-36

The examination of LD isozyme anomaly case profile in our hospital.

Mariko Watanabe[1)] Masanori Seimiya[2)] Yachiyo Endo[1)] Haruna Asano[1)]
Keiko Saito[1)] Toshihiko Yoshida[1)] Yuji Sawabe[1)] Kazuyuki Matsusita[1)]
1) Division of Laboratory Medicine, Chiba University Hospital
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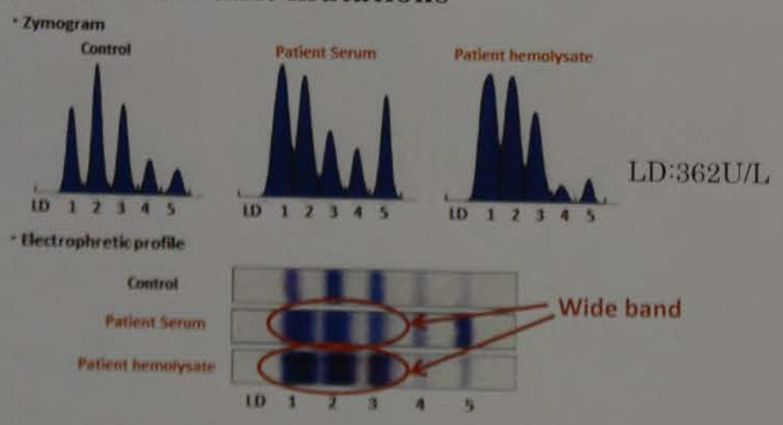
For the analysis of LD isozyme, Epalyzer and dedicated reagents were used. For anomalies, LD isoenzymes in hemolysates of patients erythrosytes were analyzed to examine gene mutations. For cases without abnormal electrophoretic image with the hemolysates, electrosyneresis or immunofixation (IFE) were conducted.

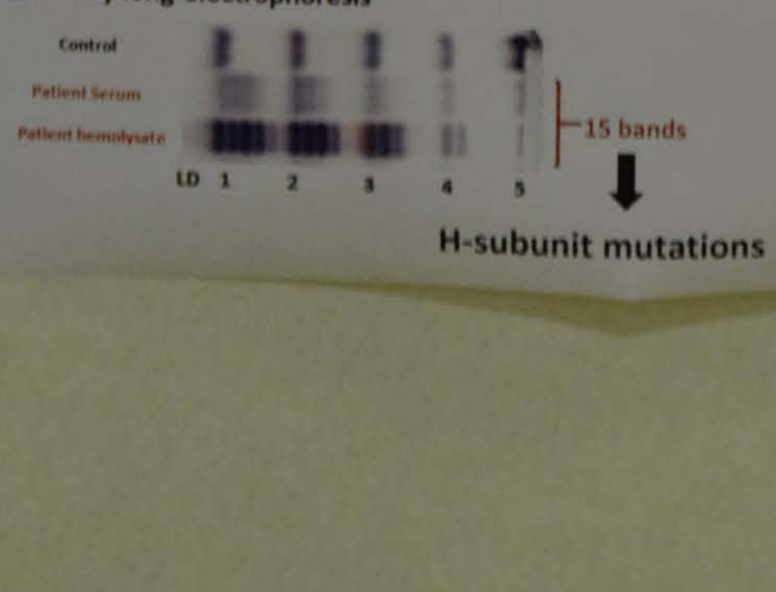
【Results】

In the past decade, 36 LD isozyme anomalies were detected, which accounted for 0.8% of all tested cases. They included genetic abnormalities (3 cases), enzyme-linked immunoglobulin (14 cases), LD6 (ALDH; 1 cases), and unknown cause (18 cases).

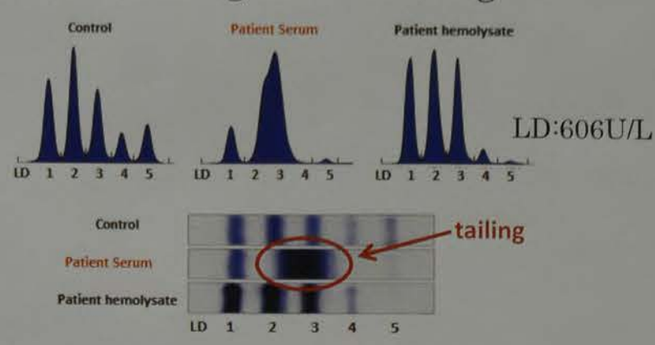
Case 1: H-subunit mutations



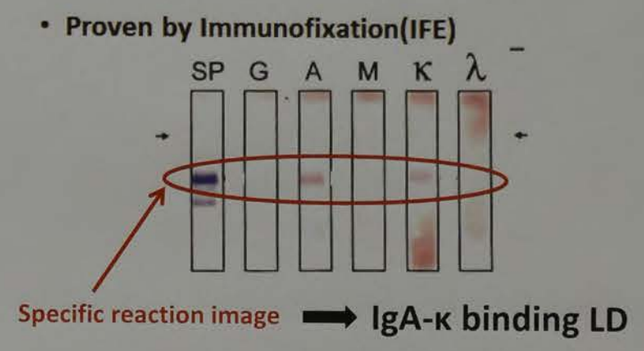
Serum and hemolysate revealed anomaly pattern.



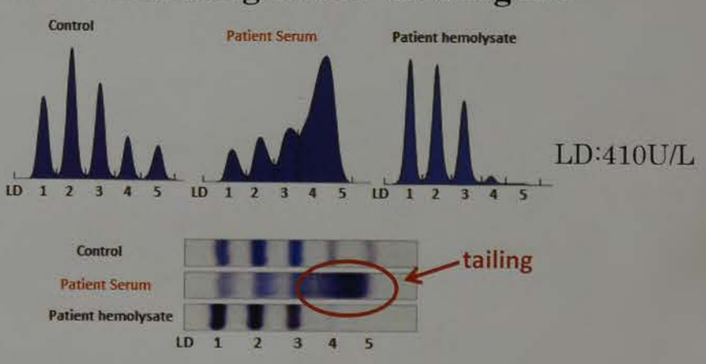
Case 2 :Immunoglobulin binding LD



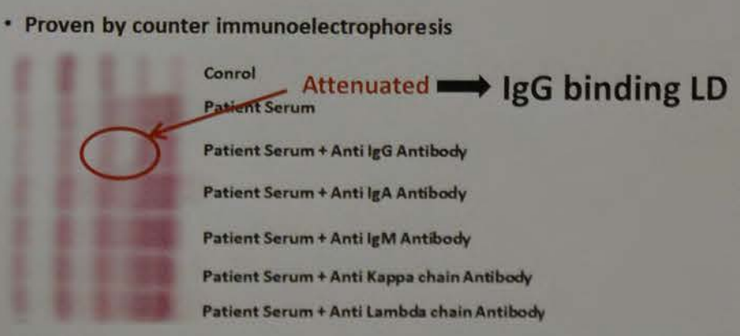
Only Serum revealed an anomaly pattern.



Case 3 : immunoglobulin binding LD



Only Serum revealed an anomaly pattern.



【Conclusions】

Total LD activities were abnormally high in all the 14 cases with immunoglobulin binding identified. This may have been caused by the extended turnover of plasma LD through binding between LD and immunoglobulin. The 18 cases of unknown cause may have been due to binding to plasma components, such as immunoglobulin, because anomalies occurred during and after their release into the blood. In these cases, abnormally high LD values may not reflect tissue injuries. Therefore, clinical laboratory scientist should clearly report phenomena to facilitate their understanding. Although it is laborious to search for causes of anomalies, they should be identified for accurate diagnosis wherever possible.

Immunology

PB-37

Identification of association of squamous cell carcinoma antigen with IgA

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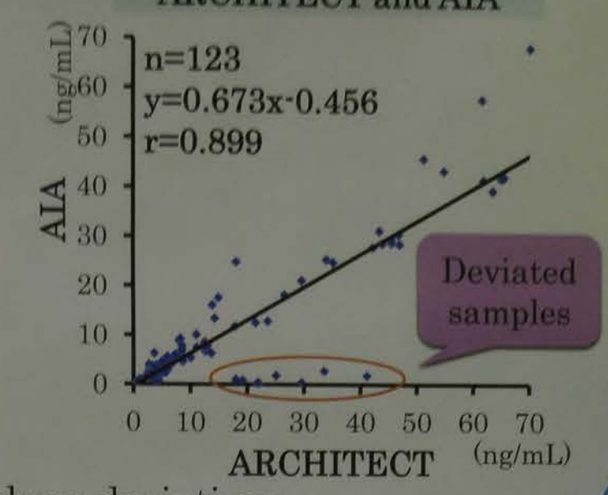
Background

Squamous cell carcinoma antigen (SCCA) is used as a tumor marker for squamous cell carcinoma and is routinely measured in clinical laboratories.

We validated two different SCCA kits.

ARCHITECT	Chemiluminescent immunoassay
AIA	Fluorescent enzyme immunoassay

We found that SCCA levels of 8 samples from three patients deviated from the correlation slope.



Aim

We aimed to elucidate the mechanisms responsible for these deviations.

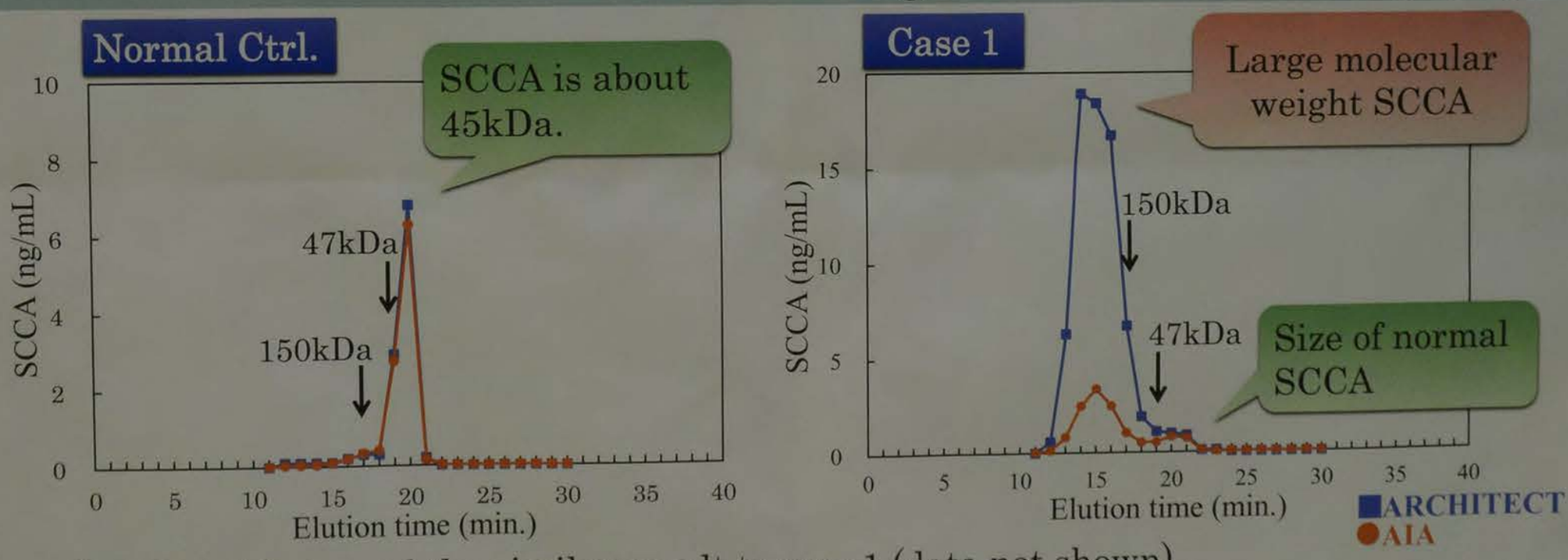
Exp. 1 Polyethylene glycol precipitation

	SCCA (ng/mL)		Recovery (%)
	Treated with PEG	Treated with H_2O	
Case 1	0.2	20.5	1.0 ↓
Case 2	0.2	11.1	1.8 ↓
Case 3	0.8	16.6	4.8 ↓
Ctrl.	11.0	15.2	72.4

Low recovery rates

A formation of large molecular complexes might have affected the SCCA levels by the two immunoassays.

Exp. 2 Evaluation of molecular weight of SCCA using size exclusion chromatography



Case2 and 3 showed the similar result to case 1 (data not shown).

- A large molecular weight SCCA existed in these three cases.
- ARCHITECT-method showed higher levels of large molecular weight SCCA compared with AIA-method.

Exp. 3 Detection of anti-SCCA antibody using spike recovery tests

A recombinant SCCA was added to patients' sera.

	Case 1	Case 2	Case 3	Ctrl.
Recovery (%)	24.2 ↓	9.2 ↓	36.4 ↓	97.0

The existence of free immunoglobulin is capable of forming an immune complex with SCCA in these patients' sera.

Exp. 4 Absorption test for immunoglobulin

To evaluate which class of immunoglobulin reacted with SCCA, each sample was incubated with anti-human immunoglobulin antibody.

	IgG	IgA	IgM
Case 1	76% ↓	91%	96%
Case 2	45% ↓	97%	100%
Case 3	60% ↓	48% ↓	96%
Ctrl.	108%	106%	105%

Summary

These results indicate that large molecular weight SCCA complexed with IgG (case1 and 2) and IgG plus IgA (case 3) existed in the sera from deviated cases.
The reason for the deviation might be due to the differences in the reactivity to SCCA immune complex with their autoantibody.
To our knowledge, this is the first report to describe the coupling of SCCA with IgA.

This study was approved by the Institutional Research Ethics Committee of the Faculty of Medicine, The University of Tokyo (Number 3333-95).

Immunology

PB-38

A case report of retroperitoneal germ cell tumor with chronic hepatitis C: an abnormal AFP fractionation was useful for diagnosis

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Abstract

Most germ cell cancer occurs inside the gonads, however five % of malignant germ cell tumors appear outside their organs. Because extragonadal germ cell tumors (EGGCTs) are found anywhere on the midline such as mediastinum and retroperitoneum, the origin of this tumor remains controversial. EGGCTs are often seen between childhood and young adult; an elderly patient with EGGCT is rarely met. Here we report a case that an abnormal AFP fractionation pattern was helpful for early diagnosis of retroperitoneal germ cell tumor.

An elderly man with chronic hepatitis C showed an intraperitoneal tumorous lesion on computed tomography and thus hepatocellular carcinoma suspected. A serological test revealed elevated total alpha-fetoprotein (AFP) level and AFP-L3 %. The latter is the proportion of glycosylated AFP based on the lectin-affinity. Noticeably the fractionation pattern of AFP of this patient was abnormal, suggesting a diversity of lectin-affinity of AFP in germ cell tumors. This patient also showed an atypical increase in beta human chorionic gonadotropin (βhCG). We propose the measurement of βhCG for early differential diagnosis of retroperitoneal germ cell tumor and hepatocellular carcinoma in the case of detecting abnormal fractionation of AFP.

Case

Case description

Male, 63 years old
Symptoms: backache and postprandial abdominal fullness
Developing non-treated chronic hapatitis C

Examination findings

<Imaging test>
Intraperitoneal tumor-like mass was shown on CT (Figure1)
PET-CT revealed abnormal accumulation of FDG in abdomen (SUVmax=8.13, Figure 2)

<Laboratory test>
Laboratory findings were indicated in Table 1
AFP fractionation pattern was abnormal because it couldn't distinguish AFP-L1 from AFP-L3 by μTASWako i30 (Wako Diagnostics). (Figure 3)

Table 1 Values of hematological, biochemical and immunological tests in the patient.

Tests	Unit	Value	Tests	Unit	Value	Tests	Unit	Value
WBC	(10^3/μL)	6.02	UA	(mg/dL)	6.3	TTT	(KU)	27
Neut	(%)	70.8	TB	(mg/dL)	1.0	ZTT	(KU)	>40
Lymp	(%)	17.1	DB	(mg/dL)	0.3	IgG	(mg/dL)	4251
Mono	(%)	6.9	AST	(U/L)	70	IgA	(mg/dL)	860
Eos	(%)	1.5	ALT	(U/L)	57	IgM	(mg/dL)	119
Bas	(%)	0.4	LDH	(U/L)	149	AFP*	(ng/mL)	4558.0
RBC	(10^6/μL)	328	ALP	(U/L)	862	AFP**	(ng/mL)	4363.8
Hb	(g/dl)	11.2	γ-GTP	(U/L)	328	AFP-L3	(%)	>99.5
Ht	(%)	33.4	CHE	(U/L)	51	βhCG	(mIU/mL)	313.8
MCV	(fl)	101.9	AMY	(U/L)	105	CEA	(ng/mL)	4.5
MCH	(pg)	34.3	CK	(U/L)	41	CA19-9	(U/mL)	85.1
MCHC	(%)	33.6	GLU	(mg/dL)	128	NSE	(ng/mL)	11.9
Plt	(10^3/μL)	129	TC	(mg/dL)	131	CA125	(U/mL)	162.5
PT	(sec)	16	TG	(mg/dL)	76	PIVKA II	(mAU/mL)	137
PT-INR	-	1.32	CRP	(mg/dL)	5.44	M2BPGi	(C.O.I.)	12.04
aPTT	(sec)	41.8	Na	(mmol/L)	136	HBsAg	(C.O.I.)	0.1
TP	(g/dL)	9.1	K	(mmol/L)	4.6	HBcAb	(C.O.I.)	4.2
Alb	(g/dL)	2.4	Cl	(mmol/L)	101	HCVAb	(C.O.I.)	74.9
BUN	(mg/dL)	15	Ca	(mg/dL)	9.6	HBV DNA	(Log copies/mL)	not detected
CRE	(mg/dL)	0.75	IP	(mg/dL)	2.9	HCV RNA	(LogIU/mL)	2.8

*:cobas8000, **:μTAS-WAKO

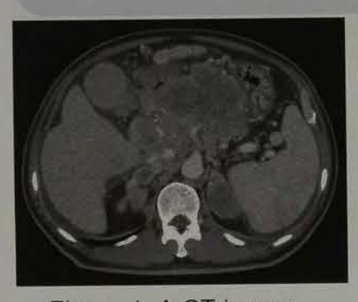

Figure 1. A CT image.

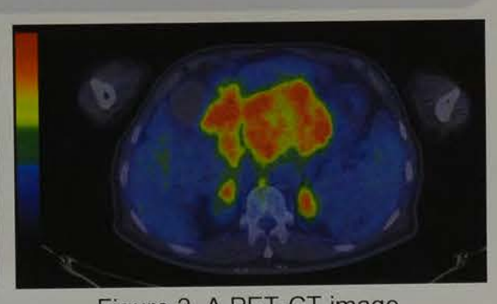

Figure 2. A PET-CT image.

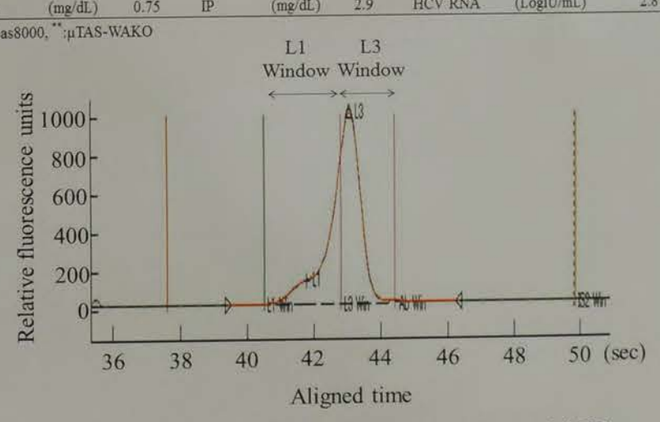

Figure 3. An abnormal fractionation pattern of AFP.

Purpose

AFP-L3 is fractionated based on affinity between glycosylated AFP and lectin. In this patient, it is unknown by what an abnormality of AFP fraction was caused. So we attempted to analyze whatever abnormal AFP fractionation was attributed to glycosylated AFP using μTASWako i30.

Result

AFP fraction pattern of the patient was difference compared to well-separated control (Figure 4)
AFP fraction showed mono-modal distribution when AFP was fractionated using buffer without lectin (LAC) (Figure 5, Lower)
This data suggests patient's AFP is glycosylated and have an affinity to lectin

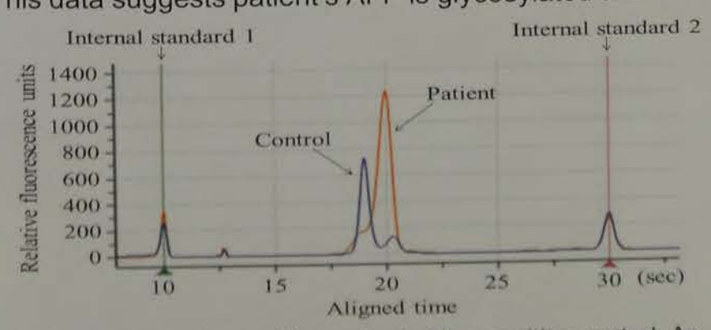

Figure 4. Comparison of the patient with a positive control. An AFP fractionation pattern of the patient (Patient) and a positive control (Control) were merged with reference to two internal standards.

Table 2 Transition of AFP, AFP-L3 and βhCG values.

Day	AFP (ng/mL)	AFP-L3 (%)	βhCG (mIU/mL)
1	4363.8	>99.5	313.8
8	4615.7	>99.5	370.1
22	6208.1	>99.5	472.4
28	6263.0	93.7	621.0
31	5166.2	93.9	739.1

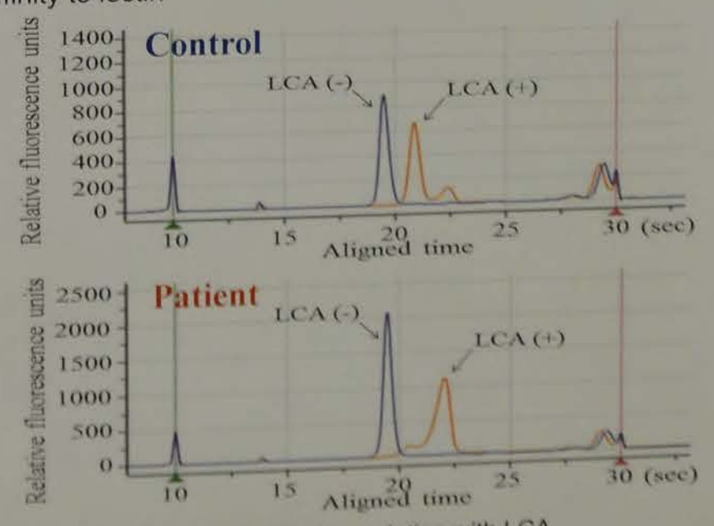

Figure 5. Evaluation of AFP glycosylation with LCA. (Upper) AFP fractionation of a positive control serum was done in the buffer containing LCA (LCA (+)) or no LCA (LCA (-)). (Lower) AFP fractionation of the patient's serum was done as in upper figure.

Discussion

An abnormality of AFP fraction was reported in the case of germ cell tumors (GCTs). It makes AFP fractionation difficult that the ratio of AFP-L2 is elevated and gives a diversity of affinity to lectin in GCT. In most extragonadal GCTs, an abnormal increase of βhCG was reported. This patient also showed high βhCG value. βhCG continued to elevate accompanied by increasing of AFP until discharge (Table 2).Because there was no evidence to suspect testicular tumor through ultrasound test, this patient was diagnosed as extragonadal (retroperitoneal) GCT.

Conclusion

We propose the measurement of βhCG for early differential diagnosis of extragonadal GCT and hepatocellular carcinoma when an abnormal fractionation of AFP was detected.

Immunology

PB-39

Defective clot retraction by Bence Jones protein in a patient with Multiple Myeloma.

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KYUSHU UNIVERSITY

Background and purpose:

A patient with multiple myeloma (MM) has M-protein, monoclonal immunoglobulin produced by increased malignant plasma cells. Bloods from patients with MM are known to show a defective clot retraction though infrequently. These defective clot retractions are caused by M-protein. The defective clot retraction is due to a lack of fibrin monomer polymerization, but its mechanism remains to be fully elucidated.

We observed a MM patient with an impaired clot retraction, whose M-protein is lambda type Bence Jones protein (BJP). In the most cases, M-protein which impairs the clot retraction is intact immunoglobulin like IgG or IgM. Thus it is extremely rare that the clot retraction is caused by BJP consisting of only a light chain dimer. Furthermore, BJP usually does not work as antibody because it is not intact immunoglobulin. This patient's BJP also may impair clot retraction without antigen-antibody reaction.

In this study, we revealed the patient' s BJP was a cause of defective clot retraction.

Patient Profile:

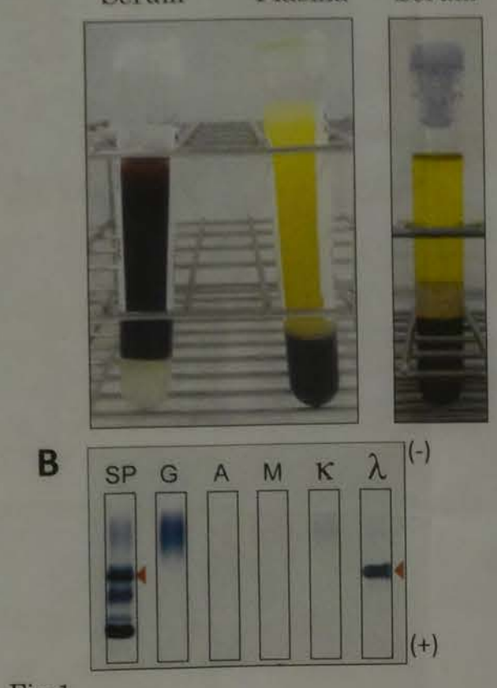

Fig 1.
A: Blood samples of the patient.
B: Immunofixation electrophoresis analysis of patient's serum

Table1. Blood test of the patient.

Biochemical findings			reference	Hemostatic findings			reference
TP	7.2	g/dL	(6.6-8.1)	WBC	2.84	x10^3/μL	(3.5-8.6)
Alb	4.1	g/dL	(4.1-5.1)	RBC	2.10	x10^6/μL	(3.86-4.92)
BUN	66	mg/dL	(8-20)	Hb	7.2	g/dL	(11.6-14.8)
Cre	5.47	mg/dL	(0.46-0.79)	Ht	21.4	%	(35.1-44.4)
Ca	12.7	mg/dL	(8.8-10.1)	MCV	101.9	fL	(83.6-98.2)
IgG	591	mg/dL	(861-1747)	MCH	34.1	pg	(27.5-33.2)
IgA	10	mg/dL	(93-393)	MCHC	33.5	g/dL	(31.7-35.3)
IgM	10	mg/dL	(33-183)	RETI	12	‰	(2-18)
A/G ratio	0.57		(1.32-2.23)	PLT	236	x10^3/μL	(158-348)

・Japanese male, 65-year-old.

・In the patient's blood sample without anticoagulant, **the clot hardly retracted and it was very difficult to separate serum**, even though his heparinized blood sample can separate plasma normally (Fig 1A).

・Patient was diagnosed as MM with blood tests indicating anemia, impaired renal function, hypercalcemia, hypogammaglobulinemia, decreased albumin-globulin (A/G) ratio (Table 1).

・Immunofixation electrophoresis analysis showed his M-protein was lambda type BJP (Fig 1B).

Result 1:

Patient's BJP shows an unusual high molecular weight type, not only a light chain dimer.

Immunoblot assay utilize anti lambda chain under non-reducing condition to prevent dissociation of lambda light chains.

In addition to intact immunogloblin and lambda light chain dimer, we identified **68kDa band as a unusual high molecular weight type** in Patient's serum but not in another patient with lambda type BJP MM (Fig 2).

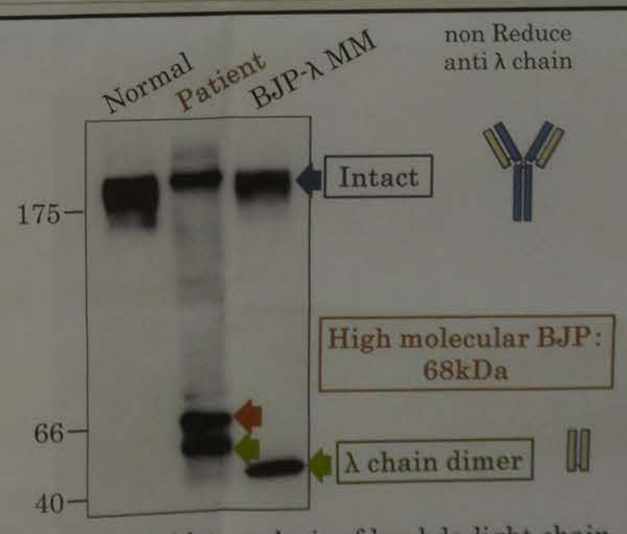

Fig 2. Immunoblot analysis of lambda light chain.

Result 2:

Patient's BJP inhibited fibrin fiber formation.

To confirm the relation between the patient's BJP and fibrin polymerization, we analyzed thrombin-induced fibrin polymerization (TIFP) assay.

After thrombin cleaves fibrinogen into fibrin, fibrin binds each other to form fibrin fiber via protofibril. Fibrin fiber, i.e. blood clot, retracts itself finally.

In TIFP assay, optical density does not increase during protofibril formation (lag time) and then begins to increase as the fibrin fiber formation (Fig 3A).

The lag time of normal plasma was prolonged from 140 sec to 250 sec by purified patient' s BJP of this patient but not by other patients' lambda type BJP (Fig 3B).

These results indicated **the patient' s BJP inhibited fibrin fiber formation** (Fig 3C).

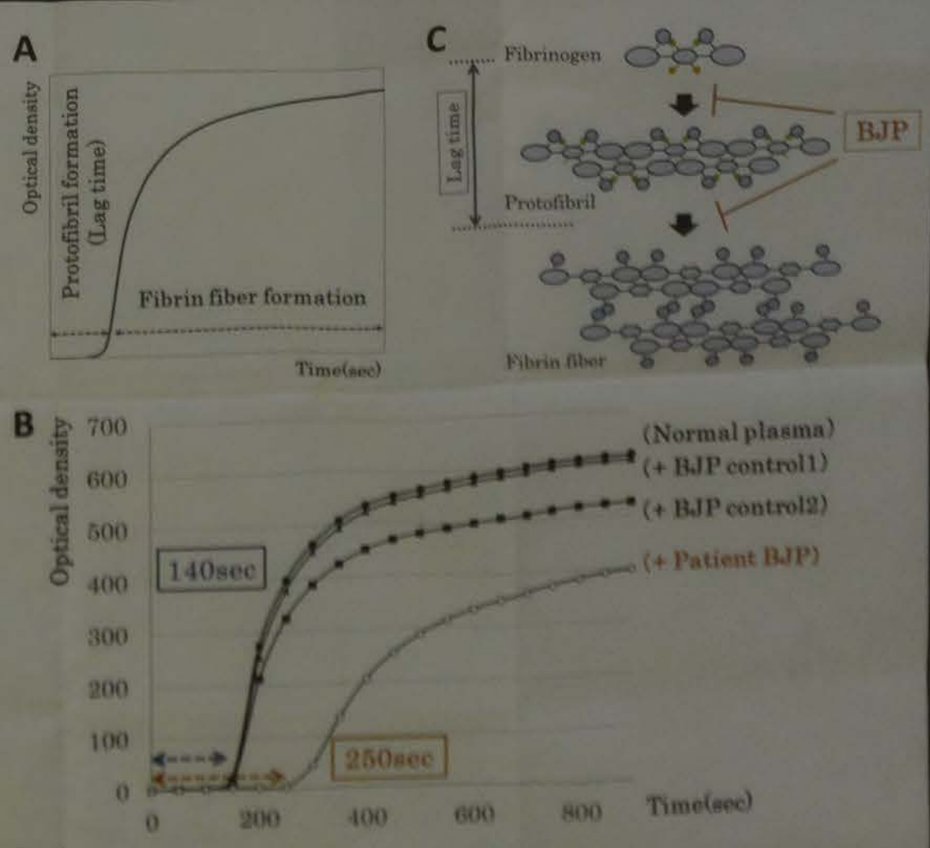

Fig 3.
A: Thrombin-induced fibrin polymerization (TIFP) can monitor fibrin fiber formation.
B: Patient's BJP prolonged the lag time.
C: The schema of inhibition of fibrin fiber formation by patient's BJP.

Conclusions:

In the current study, the patient's BJP showed not only light chain dimer, but also unusual high molecular type BJP. We also reveal that the purified patient's BJP inhibited fibrin fiber formation.
Because BJP is not intact immunoglobulin, it is considered not to have an antibody activity. Therefore, the patient's

Immunology

PB-40

Discrepancy between serum agarose gel electrophoresis and immunofixation pattern: An analysis of a case of M-proteinamia

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1) Department of clinical laboratory, Japanese for cancer institute Ariake Hospital
2) Department of clinical laboratory medicine, Juntendo University, school of medicine

【Introduction】

The number of the M protein bands detected on immunofixation electrophoresis (IFE) generally shows high concordance with the number of "M-peaks" on serum protein agarose gel electrophoresis (AGE). We herein report a case of M-proteinemia which showed a different number of M-protein bands between IFE and AGE and investigated the cause of this discrepancy.

【Patient】

The case was a 74-year-old woman diagnosed with solitary plasmacyotma of the thoracic vertebra. A serum analysis on first admission showed two "M-peak" bands in the γ-globulin region on AGE (Figure1) but only one M-protein (IgG-λ) band on IFE (Figure2). The other laboratory test results is shown in Table 1, and there are no significant serum free light chain (FLC), nor Bence Jones protein on urine IFE (Figure 3).

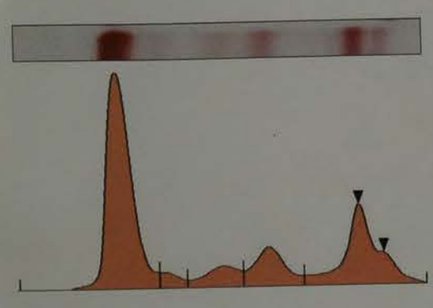

Figure1. AGE(patient serum)

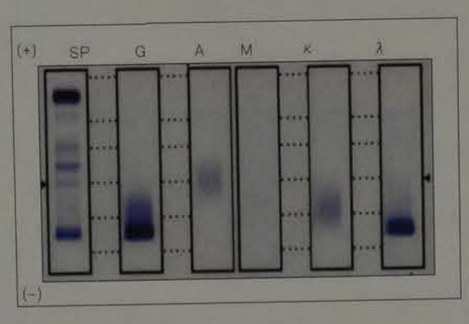

Figure2 .IFE(patient serum)

Blood data		Serum data			
WBC (×10⁶/L)	6.0	ALB (g/dL)	4.2	Na (mEq/L)	142
RBC (×10⁹/L)	3.95	T.Bil (mg/dL)	0.5	K (mEq/L)	4.9
Hgb (g/dL)	12.2	γ-GTP (U/L)	30	Cl (mEq/L)	107
Hct (%)	36.8	ALP (U/L)	130	IgG (mg/dL)	1612
MCV (fL)	93.2	LDH (U/L)	162	IgA (mg/dL)	155
MCH (pg)	30.8	AST (U/L)	27	IgM (mg/dL)	107
MCHC (g/dL)	33.0	ALT (U/L)	17	FLC-κ (mg/L)	8.0
PLT (×10⁶/L)	140	GLU (mg/dL)	97	FLC-λ (mg/L)	21.9
		Ca (mg/dL)	10.3	κ/λ	0.37
		UN (mg/dL)	16		
		CRE (mg/dL)	0.55		

Table 1. Laboratory data on the first admission

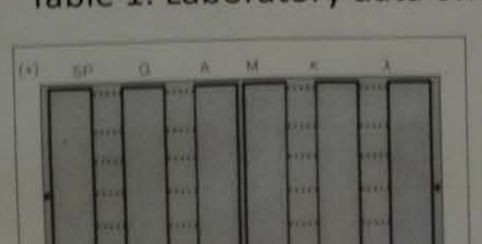

Figure3. urine IFE

【Electrophoretic Assay Materials】

All of the electrophoretic analyses were performed using an Epalyzer 2™ automated electrophoresis system (Helena Laboratories, Tokyo, Japan)

<Assay kit>

AGE gel kit: Quick Gel™: 1% agarose gel with CAPSO buffer, pH 10.2 (Helena Laboratories, Tokyo, Japan)

IFE gel kit: Quick Gel IFE™; 1% agarose gel with tris buffer, pH 9.0 (Helena Laboratories, Tokyo, Japan)

【Methods】

1. IFE using AGE gel instead of an IFE gel
2. AGE using IFE gel instead of an AGE gel
3. IFE of the pretreated sera with neuraminidase, dithiothreitol (DTT), or 2-mercaptoethanol (2ME)
   1) neuraminidase: 2U/L neuraminidase : serum＝1:3
   2) DTT(1) ; 30mM DTT : serum = 1 : 3
   3) DTT(2) ; 10mM DTT : serum = 1 : 1, 37 ˚C, 30 min
   4) DTT(3) ; 0.5M DTT : serum = 1 : 10, 25 ˚C, 30 min
   5) 2ME(1) ; 0.1M 2ME : serum = 1 : 6, 37 ˚C, 2 hours
   6) 2ME(2) ; 0.1M 2ME : serum = 1 : 1, 37 ˚C, 2 hours

【Result】

1. Figure4: two M-protein bands
2. Figure5: single M-protein bands
3. 1)Figure6-② 2)Figure6-③ 3)Figure-④ 4)Figure6-⑤ 5)Figure6-⑥ 6)Figure-⑦ No remarkable affect

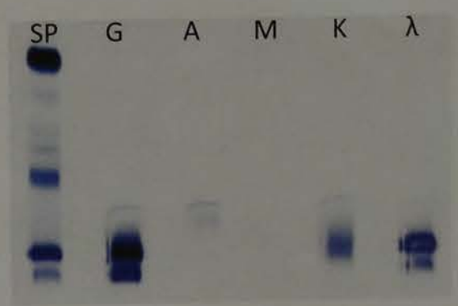

Figure4. IFE using an AGE gel. Two M-protein bands were observed

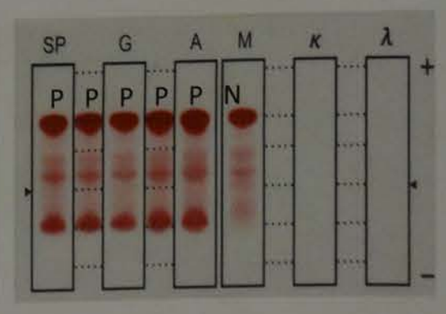

Figure5. AGE using an IFE gel.
P: patient serum
N: normal serum

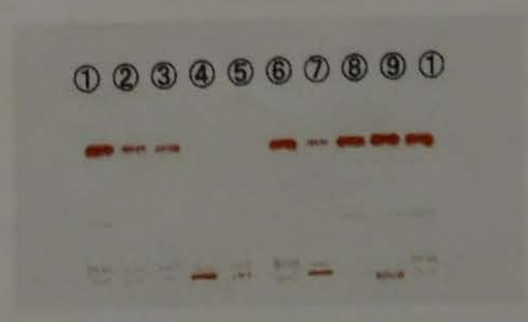

Figure6. IFE of the pretreated serum
① patient serum (no pretreatment)
② neuraminidase ③ DTT(1)
④ DTT(2) ⑤ DTT(3)
⑥ ME(1) ⑦ ME(2)
⑧ normal serum
⑨ abnormal serum

【Conclusion】

Based on these results, we concluded that the discrepancy was caused by an interaction between the isoelectric point of the M-proteins and the pH of the buffer. The AGE and IFE methods utilize the same gel material (1% agarose) but different buffers, which may have caused the observed discrepancies in the findings.