Hematology

PC-01

Plasma Cell Leukemia: A Retrospective Analysis of 14 Cases from a Single Institute in Taiwan

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Introduction

Plasma cell leukemia (PCL) is a rare and aggressive neoplasm comprising leukemic plasma cells. PCL may present at the time of initial diagnosis (primary PCL) or evolve as a late feature of plasma cell myeloma (secondary PCL). PCL has not been reported from Taiwan yet. In this study, we aimed to characterize the clinicopathological and immunophenotypic features of PCL in Taiwan.

Materials and Methods

We retrospectively searched for PCL in our institution (Chi-Mei Medical Center, Tainan, Taiwan) from year 2002 to 2015. We reviewed the medical charts for clinical features. Wright Giemsa stain was used for staining of the peripheral blood smear. We used three-color flow cytometric analysis for immunophenotype of the neoplastic plasma cells in PB. Flow cytometric results were collected and analyzed.

Results

1. We identified 14 cases including 8 cases of primary and 6 cases of secondary PCL.
2. The frequency of leukemic change was 2.5% (6/240) among patients with plasma cell myeloma during this period.
3. The 14 patients were mostly males with a M:F ratio of 6:1. The median age was 70 (range, 43-90).
4. Nearly all patients had anemia (100%; 14/14), thrombocytopenia (100%; 14/14), and impaired renal function 93%; 13/14).
5. Table 1 lists the laboratory findings of the patients.
6. Figure 1 depicts the representative images of the peripheral blood smears.
7. For flow cytometric immunophenotyping, the CD45 non- or dim-expressors were gated. All cases expressed surface (13/13) and cytoplasmic (14/14) CD38, and cytoplasmic CD138 (14/14). All cases were monotypic for light chain expression with kappa light chain restriction more common (71% or 10/14).
8. Table 2 summarizes the immunophenotypic features. The only immunophenotypic difference between primary and secondary PCL was consistent CD56 expression in the secondary cases (50% or 4/8 in primary vs. 100% or 6/6 in secondary cases; p= 0.04, Chi-square).
9. Follow-up data showed that all six patients with secondary PCL passed away in one month after leukemic change. Of the 8 cases with primary PCL, two were alive (1 and 10 months, respectively) and the remaining 6 died of disease within 1 month (4 patients), 4 months (1), and 8 months (1), respectively.

Table 1. Laboratory findings of patients with PCL.

Case no	Age	Sex	WBCx10³/ul	Hb g/dL	PLTx10³/ul	PC % in PB	PC (x10³/ul) Absolute count	Ig	Serum Ca mg/dL	Serum Cr mg/dL	β2-M mg/dL	LDH IU/L
1	74	M	4.2	10.4	131	21	0.9	IgG	NA	2.1	NA	NA
2	70	M	3.4	8.8	9	30	1.0	NA	NA	1.9	NA	NA
3	72	M	20.7	9.6	112	28	5.8	NA	8.4	5.6	NA	NA
4	90	M	19.4	11.1	135	38	7.4	IgA	10.7	1.9	NA	421
5	70	M	8.1	7.2	14	96	7.8	IgG	6.3	4.3	5117	NA
6	77	F	21.7	10.6	60	36.5	7.9	NA	8.6	5.5	NA	NA
7	83	M	51.3	10.0	32	10	5.1	NA	6.9	2.1	5090	774
8	69	M	2.6	5.7	15	38	0.9	IgA	NA	1.3	7154	NA
9	43	M	7.1	11.5	35	43	3.0	IgG	7.0	1.5	NA	1138
10	64	N	5.0	10.1	79	28	1.4	IgG	7.9	2.6	30200	124
11	63	M	41.0	8.5	57	49	20.1	NA	10.6	4.3	24000	NA
12	59	M	10.2	9.3	66	42	4.2	IgG	11.7	6.5	17500	NA
13	80	M	6.5	7.0	38	5	0.3	IgA	8.2	1.6	15184	237
14	82	F	2.5	10.1	71	8	0.2	IgG	7.9	0.9	4990	NA

Abbreviations: β2-M, β2-microglobulin; Ca, calcium; Cr, creatinine; Hb, hemoglobin; Ig, immunoblobulin; NA-Not available; PLT, platelets; PC; plasma cell; WBC, white blood cell.

Table 2. Comparison of immunophenotypic features of primary vs. secondary PCL.

	CD45	CD19	cCD19	CD38	cCD38	CD43	CD56	cCD138	Light chain
Primary	50%	14%	86%	100%	100%	86%	50%	100%	Kappa: 6
(n=8)	(4/8)	(1/7)	(6/7)	(8/8)	(8/8)	(6/7)	(4/8)	(7/7)	Lambda: 2
Secondary	50%	17%	80%	100%	100%	80%	100%	100%	Kappa: 4
(n=6)	(3/6)	(1/6)	(4/5)	(5/5)	(6/6)	(4/5)	(6/6)	(2/2)	Lambda: 2
Total	50%	15%	83%	100%	100%	83%	72%	100%	Kappa: 10
(n=14)	(7/14)	(2/13)	(10/12)	(13/13)	(14/14)	(10/12)	(10/14)	(9/9)	Lambda: 4
P value	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	0.04	>0.5	

Chi-square test

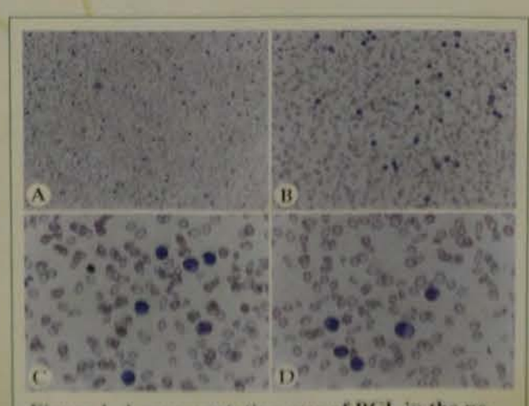

Figure 1. A representative case of PCL in the peripheral blood at various magnification (A, x200; B, x400; C & D, x1000)

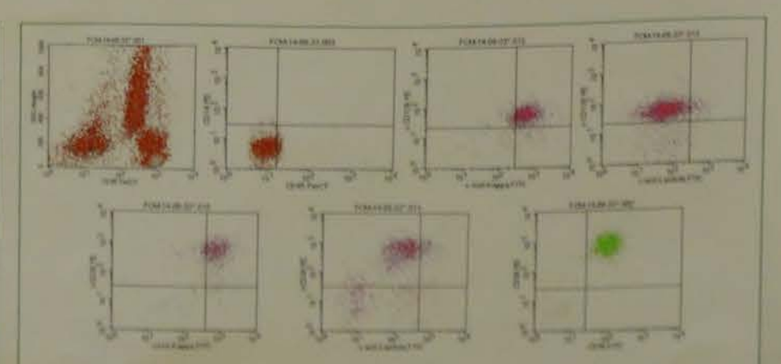

Figure 2. Flow cytometric immunophenotyping of a representative case showing neoplastic plasma cells expressing cytoplasmic CD38, cytoplasmic CD138, cytoplasmic kappa light chain and surface CD56 but not cytoplasmic lambda light chain.

Conclusion

In conclusion, we presented the clinical and immunophenotypic features of PCL in Taiwan. As compared to primary PCL, secondary cases seem to carry a higher rate of CD56 expression and a worse prognosis. A larger national wide study is warranted.

Hematology

PC-02

Leukemic Phase of Follicular Lymphoma: A Retrospective Study from a Single Institution in Taiwan

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INTRODUCTION

Follicular lymphoma (FL) is a malignant B-cell lymphoma of follicular center B-cell origin, usually has at least a partially follicular pattern. FL usually involves lymph nodes, but also spleen, bone marrow, and peripheral blood and Waldeyer's ring. FL accounts for around 22% of lymphoma cases in the West, while the frequency is much lower in the East. In Taiwan, although the incidence is increasing in recent years, the frequency of FL among all non-Hodgkin lymphoma is around 14%. FL with leukemic change is uncommon and has not been well characterized and has not been reported from Taiwan before.

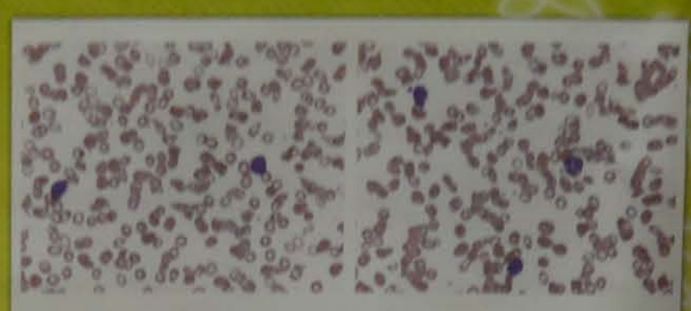

Figure 1.
Representive images of FL with leukemic change.

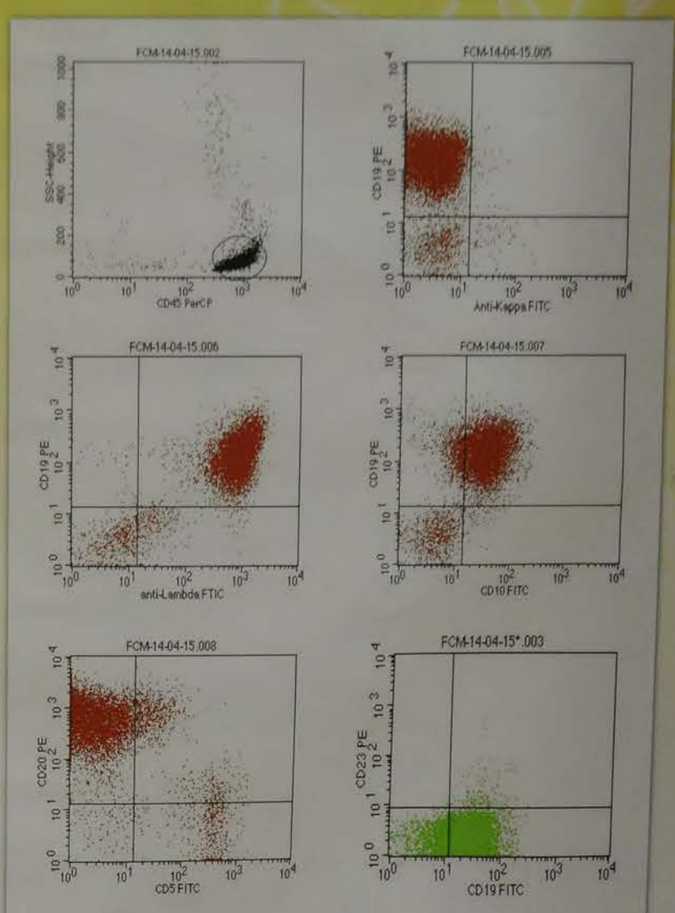

Figure 2.
A representative case of leukemic FL from peripheral blood. Flow cytometric immunophenotyping shows leukemic cells express CD10, CD19, and CD20 with monotypic lambda light chain expression but not CD5, CD23, or kappa light chain.

MATERIALS AND METHODS

We retrospectively investigated leukemic FL in our institution from January 2000 to December 2015. We reviewed the clinicopathological features and flow cytometric immunophenotyping (three-color with CellQuestPro from Becton Dickinson)

RESULTS

1. **During this period, there were a total of 168 cases of FL. Among them, 10 (6.0%) cases experienced leukemic change, either at presentation (6 cases) or at disease course (1, 3, 20, or 26 months after initial diagnosis).**
2. **The 10 patients included four males and six females with a median age of 47.5 (range, 30-75).**
3. **The original nodal lymphomas were low-grade in eight cases (seven grade 1 and one grade 2) and high-grade in 2 cases (both grade 3A).**
4. **Results of immunophenotyping: Expression of CD19 (10/10, 100%), CD23 (3/8, 33%), CD10 (2/10, 20%) and CD43 (1/7, 14%) but not CD5 (0/10, 0%).**
5. **Among the 6 cases of CD10-posititve nodal FL with leukemic change, the leukemic cells lost CD10 in 4 cases.**
6. **Of the 165 cases of FL, CD10 was expressed in 121 (73%) tumors. There was no significant difference in terms of CD10 expression in cases with or without leukemic change (6/10 or 60% vs. 115/155 or 74.2%; p= 0.459, Fisher exact test).**
7. **The patients were treated with chemotherapy. Follow-up data showed that six patients were alive including five who were free of disease (median 51 months; range, 28-111) and one who was alive with disease (19 months). The remaining four patients died of disease at 5, 23, 55, and 61 months, respectively, after leukemic change.**

CONCLUSION

In this study, we reported the clinicopathological and immunophenotypical features of FL with leukemic change in Taiwan. We found that the frequency of CD10 expression in leukemic FL was relatively low as compared to nodal FL (121/165 or 73% vs. 2/10 or 10%; p <0.001, Fisher's exact test), which might be due to different epitopes for detection in immunohistochemistry and flow cytometry. Alternatively, loss of CD10 expression might play a role in the pathogenesis of leukemic change. The clinical course of leukemic FL could be aggressive, but a significant proportion of the patients obtained complete response with chemotherapy. Studies on larger number of patients are warranted for a better understanding of the impact of leukemic change in patients with FL.

Hematology

PC-03

How Much Sample is Enough for Complete Blood Count ? Evaluation of the Accuracy of Complete Blood Count for Insufficient Blood Samples

PC-03

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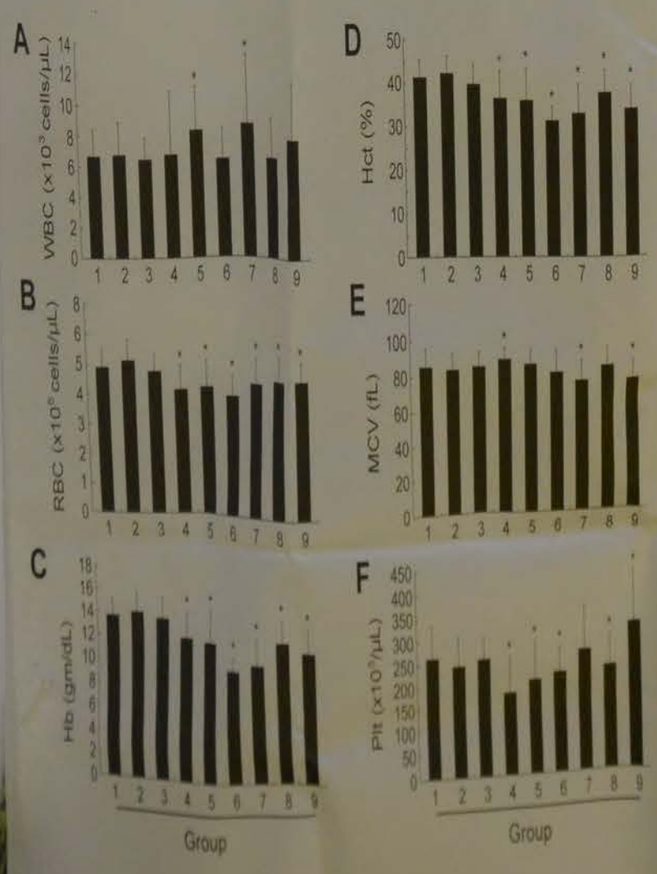
Introduction Complete blood count (CBC) is one of the most common laboratory studies in different clinical settings. It usually happens that standard blood sample volume of 3 mL cannot be obtained. This study aimed at investigating the impact of diminished blood sample volumes on the accuracy of CBC analysis.

Patients and Methods Between August 1 and December 31, 2010, 332 blood samples from 332 subjects from a single medical institute were divided into nine groups according to clinical settings, including new employees (Group 1, n=39), individuals for physical check-up (Group 2, n=39), patients scheduled for hospitalization (Group 3, n=30), liver transplant patients (Group 4, n=48), patients undergoing hemodialysis (Group 5, n=39), patients in emergency department (Group 5, n=35), adult inpatients (Group 7, n=40), adult outpatients (Group 8, n=34), and pediatric outpatients (Group 9, n=28). Ten milliliters of peripheral blood sample obtained from each subject was aliquoted into 3, 1, 0.6, and 0.3 mL, each placed inside a separate collection tube containing K2 ethylenediaminetetraacetic acid (EDTA). Parameters of CBC obtained from 1, 0.6, and 0.3 mL samples were compared with those acquired with 3 mL sample that served as standards. Percentage bias for each parameter was compared with the criteria for within-subject biological variation (CVw).

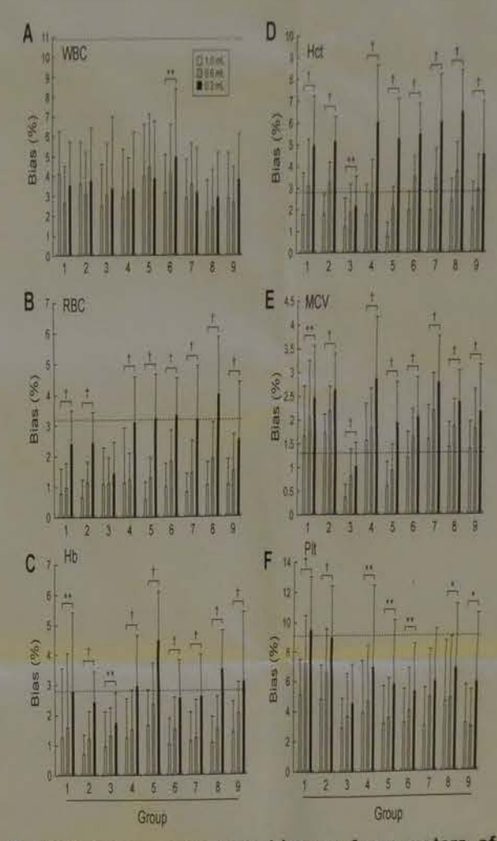
Results Significant variations, which existed among samples from different clinical settings (Fig. 1), did not notably interfere with data interpretation from small-quantity blood sample CBC analysis.



↑**Figure 1 Variations in parameters of complete blood count in different clinical settings.** WBC: White blood cell count; RBC: Red blood cell count; Hb: Hemoglobin concentration; Hct: Hematocrit; MCV: Mean corpuscular volume; Plt: Platelet count. Group 1: Newly recruited employees of the hospital without history of known systemic disease (n = 39); Group 2: Individuals without known systemic disease for annual physical check-up (n = 39); Group 3: Patients scheduled for elective hospitalization (n = 30); Group 4: Patients after liver transplantation (n = 46); Group 5: Patients with end-stage renal disease undergoing hemodialysis (n = 39); Group 6: Patients visiting the emergency department (n = 35); Group 7: Adult inpatients n = 40; Group 8: Adult outpatients (n = 34); Group 9: Pediatric outpatients (n = 28). Data expressed as mean ± SD. *p <0.05 vs. Group 1

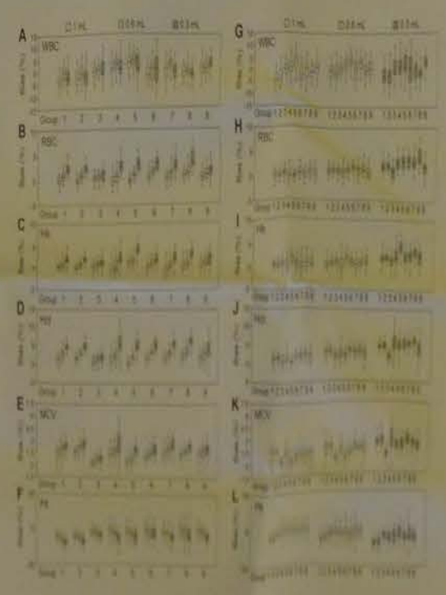
Introduction Complete blood count (CBC) is one of the most common laboratory studies in different clinical settings. It usually happens that standard blood sample volume of 3 mL cannot be obtained. This study aimed at investigating the impact of diminished blood sample volumes on the accuracy of CBC analysis.

Additionally, percentage bias for most CBC indices significantly increased with decreases in sample volume (Fig. 2). Moreover, white count was particularly unaffected by diminished blood sample volume, whereas hematocrit (Hct) and mean corpuscular volume (MCV) were vulnerable to sample volume reduction (Fig. 2).



↑**Figure 2 Changes in percentage biases of parameters of complete blood count with decreases in blood sample volume in different clinical settings.** Dotted lines representing criteria for within-subject biological variation (CVw): WBC <10.9%, RBC <3.2%, Hb <2.8%, Hct <2.8%, MCV <1.3%, Plt <9.1%. *p <0.05, **p <0.01, †p <0.001 within the same group determined by p for trend.

Furthermore, decreases in median percentage biases with reduced sample volumes were only observed in platelet count but did not significantly affect the validity of data interpretation (Fig. 3).



↑**Figure 3 Changes in median percentage biases of parameters of complete blood count with decreases in blood sample volume in different clinical settings.** Changes in median percentage biases of parameters of complete blood count with decreases in blood sample volume within the same group (A-F) and for each volume among different groups (G-L).

Conclusions: Small volumes of blood samples conventionally considered insufficient may produce valid data for interpretation for most CBC indices. Dilution effect occurred only in platelet count but did not affect data validity. The findings, therefore, may validate clinical use of small-quantity sample for CBC analysis.

Hematology

PC-04

Cell Yield of Cerebrospinal Fluid Cell Count Using Cytospin-3 and Cytopro-7620

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BACKGROUND

- The cells are concentrated approximately 20-fold by cytocentrifugation.
  Even hypocellular samples with a hematocytometer cell count of 0 cell/mcL can have a yield of approximately 35 cells on slide (CLSI H56-P).
- This study evaluated the nucleated cell number for cells recovered on slide by using CYTOPIN-3 (Thermo Shandon Ltd., UK) and CYTOPRO-7620 (Wescor Inc., USA) cytocentrifuges to hematocytometer cell count 0-5 WBCs/mcL of hematocytometer in the cerebrospinal fluid cell count.
- Next, this study aimed to examine whether the cell yield was useful for slide preparation and retest criteria.

Methods

Material

148 samples of 0-5 WBCs/mcL on hematocytometer, were cytocentrifuged by CYTOPSIN-3 and CYTOPRO-7620 instruments.

Cell count procedure

Neubauer hematocytometer, Cover glass (20x26x0.4 mm), Calibrated autopipet, CSF 20 mcL, Duplicate counting

Differential count procedure (Fig. 1-2)

- CYTOSPIN-3 ; 250 mcL in cytofunnel/filter card/cytoclip
- CYTOPRO-7620 ; 200 mcL in cytofunnel/cytopad
- Cytocentrifugation ; 700 rpm/ 5 min

Fig 1. Cytopsin-3 cytocentriguge (left), Cytopro-7620 cytocentriguge (right)

Fig 2. Cytopsin-3 sample chamber (left), cytopro-7620 sample chamber (right)

Cytoslide preparation (Fig. 3)

- CYTOSPIN-3 ; 6 mm round circle
- CYTOPRO-7620 ; 7 mm round circle
- Wright Stain ; Methanol 5 sec, Wright 5 min, Mixture (Wright 1 + PBS 4) 8 min 30 sec, PBS 15 sec, Tap water 5 sec, Dry 3 min
- Cell count & Differential count observation ; 200X, 400X
- Cell yield ; 100X, 200X

Fig 3. Wright stained CYTOSPIN-3 slide demonstrating a cell concentrated area (coated slide, plus charge, cerebrospinal fluid containing very few cells, left), Wright stained CYTOSPIN-3 slide demonstrating a cell concentrated circle (uncoated slide, center), Wright stained CYTOPRO-7620 slide demonstrating a cell concentrated circle (uncoated slide, right)

Results

- Cell yield by CYTOSPIN-3
  The nucleated cell number for cells recovered on slide was 0-40 cells in the 22 samples of 0 WBC/mcL, and 4-95 cells in the 18 samples of 1 WBC/mcL. It was observed that the nucleated cell number for cells recovered on slide was 16-100 cells in the 20 samples of 2 WBCs/mcL, and more than 100 cells in the 15 samples of 3-5 WBCs/mcL, respectively **(Table 1)**.

Table 1. Expected cell yield by Cytospin-3 cytocentrifuge

0-5 WBCs/mcL on hematocytometer (n=75)	Nucleated cell number for cells recovered on slide (cells)			
	Range	Lower Value	Median Value	Upper Value
0 WBC/mcL (n=22)	0-40	0	7	40
1 WBC/mcL (n=18)	4-95	4	53	95
2 WBCs/mcL (n=20)	16-100	16	63	100
3-5 WBCs/mcL (n=15)	≥100	≥100	-	-

Abbreviation: WBC, white blood cell.

- Cell yield by CYTOPRO-7620
  The nucleated cell number for cells recovered on slide was 0-23 cells in the 22 samples of 0 WBC/mcL, and 3-44 cells in the 13 samples of 1 WBC/mcL. It was observed that the nucleated cell number for cells recovered on slide was 13-100 cells in the 24 samples of 2 WBCs/mcL, and more than 100 cells in the 14 samples of 3-5 WBCs/mcL, respectively **(Table 2)**.

Table 2. Expected cell yield by Cytopro-7620 cytocentrifuge

0-5 WBCs/mcL on hematocytometer (n=73)	Nucleated cell number for cells recovered on slide (cells)			
	Range	Lower Value	Median Value	Upper Value
0 WBC/mcL (n=22)	0-23	0	1	36
1 WBC/mcL (n=13)	3-44	3	24	44
2 WBCs/mcL (n=24)	13-100	13	55	100
3-5 WBCs/mcL (n=14)	≥100	≥100	-	-

Abbreviation: See Table 1.

- In addition, extremely normal lymphocyte, monocyte and polymorphonuclear neutrophil were observed in the 143 samples of 0-5 WBCs/mcL. Macrophage and eosinophil were also rarely observed **(Fig. 4)**.

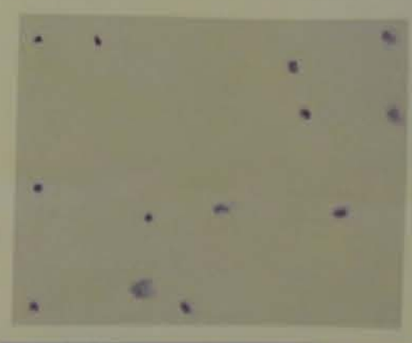

Fig 4. Cytocentrifuge slide prepared from a cerebrospinal fluid (3 WBCs cell count run for 5 min at 700 rpm, ≥100 WBCs cell yield, normal lymphocytes and monocytes, Wright stain, 200X)

Conclusion

- The nucleated cell number for cells recovered on slide was 20 cells, Which were regarded as 1 WBC/mcL in body fluid cell count. However, In this study, we made alterations to report nucleated cell percentage as 0% without preparing the cytocentrifuged slide at 0 WBC/mcL by using the cell yield in a comparison between the value of 0-5 WBCs/mcL and nucleated cell number for cells recovered on slide.
- Moreover, we slide compared with WBC number, and repeated the preparation with a new slide when sufficient WBCs were not observed in Wright stained slide.

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Hematology

PC-05

Reference intervals of mean platelet volume and platelet mass index in neonate from Taiwan

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Introduction

Mean platelet volume (MPV) is an important risk factor for predicting cardiovascular events, such as stroke and acute myocardial infarction, in adults. In addition, it is increased in diabetes, obesity, as well as rheumatologic and systemic diseases, including psoriasis and familial Mediterranean fever. MPV has also been investigated in some of the patients with platelet related disorders, and maternal MPV is associated with an adverse neonatal outcome.

Normal reference intervals of MPV and platelet mass index (PMI) show variation from region to region and between different ethnic groups. This descriptive cross-sectional study aimed at establishing the reference intervals of MPV and PMI for preterm and term infants from Taiwan.

Materials and Methods

The study was conducted at our hospital from May 2015 to February 2016. We collected 205 neonates on post-natal day 1. A 0.5-mL blood sample was collected in the tube containing K_3EDTA as an anticoagulant. Complete blood count including MPV was performed by Sysmex XE-5000 analyzer within 120 minutes after venipuncture. Preterm group (gestational age (GA) ≤ 36 weeks, n=81) and term group (GA > 37 weeks, n=124) were divided. We collected the demographic data, including GA, birth body weight (BBW), and platelet count, as well as analyzed the MPV and PMI between two groups.

Table 1. The comparision of term and preterm about MPV and PMI

	Term(n=124)	*Preterm(n=81)*	*P value*
Gestational age(week)	38.6 ± 1.1	33.4 ± 2.8	< 0.01
Birth body weight(g)	3053.7 ± 439.5	2112.8 ± 579.5	< 0.01
Platelet count(10^9/L)	249 ± 51	244 ± 65	0.601
MPV(fL)	9.7 ± 0.6	10.0 ± 0.6	0.002
PMI	2471.6 ± 552.9	2425.7 ± 477.8	0.533

MPV: mean platelet volume, PMI: platelet mass index

Results

The GA was 38.6 ± 1.1 weeks in term group and 33.4 ± 2.8 weeks in preterm group. The BBW was 3053.7 ± 439.5 gm in term group and 2112.8 ± 579.5 gm in preterm group. The platelet count was 249 ± 51 x 10^9/L in term group and 244 ± 65 x 10^9/L in preterm group. The Reference intervals of MPV and PMI in newborn are 9.8 ± 0.6 fL and 2474.7 ± 524.4, respectively. There is a significant higher MPV in preterm group than that in term group (10.0 ± 0.6 fL and 9.7 ± 0.6 fL, respectively, p = 0.002). However, there is no significant difference in PMI (2425.7 ± 477.8 and 2471.6 ± 552.9, respectively) (Table 1).

Discussion

Analysis of MPV and PMI is a simple and really available laboratory test. Recently, there are substantial studies discussing the associations between MPV and neonatal morbidity, as well as between PMI and neonatal morbidity. However, there are no reference intervals of MPV and PMI defined for Taiwan neonates. The study is the first report of the reference intervals. We expect that we could figure out the correlations of MPV and PMI with neonatal morbidity, such as sepsis and hemodynamically significant patent ductus arteriosus, in the near future.

Conclusion

Our results provide the reference intervals for MPV and PMI of newborns in Taiwan. There is a higher MPV in preterm than that in term group. Nevertheless, no difference is observed on PMI between two groups.

References

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Happiness

專業・熱忱・服務 健康・希望・幸福

Production of High Quality Specimen for Her
Diseases on Bone Marrow Examina

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und : Recently the diagnosis of hematologic diseases cou
ripheral blood due to cytogenetic chromosome analysis, i
stry, flow cytometry, FISH and development of molecular
bone marrow examination is still compulsory for definite
tological diseases such as hematologic cancer and m
neoplasms. Many medical institutions conduct bone mar
with medical technologists jointly, who must judge the a
one marrow specimen harvested by doctors. Therefore,
gest for high quality bone marrow smear slide making
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s and methods : This study made success cases of bon
the adult patients having bone marrow examinationa
of C university hospital in Korea and bone marrow was har
erior superior iliac crest and used Jamshidi needle. Biopsy
rint was made, and it was put in formalin solution of 10
partment of Pathology, prepared with paraffin section
ion with Wright staining slide. When bone marrow a
en was coagulated and biopsy specimen was not suitable
slide using cytospin centrifuge and implemented Wrigh

In case that bone marrow aspiration was failed, we imp
tain of a well made touch print, and as a result of obser
pe, we could be possible bone marrow cell differential
of cell morphology. When we did not have bone marrow
due to dry tap etc. in case of bone marrow aspiration,
block using bone marrow biopsy specimen, and imple
ain using the paraffin section. As a result of estimati
gy using microscope, we accomplished effectively bone
ential count and estimation of cell morphology and cell
at we calculated bone marrow cell differential count of
Vright stain was completed using microscope, the effec
ell differential count and estimation of cell morphology
, and report of the result was succeeded.

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Hematology

PC-06

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Defining automated ESR analyser daily quality control procedure by retained patient sample

Chen Mei-Chih

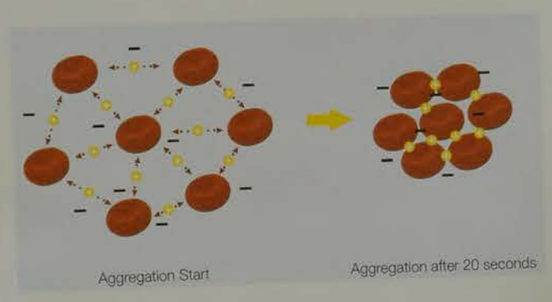
Taipei Tzu Chi Hospital, Buddhist Tzu Chi Medicine Foundation, Department of Laboratory Medicine, New Taipei City Taiwan

Introduction

The erythrocyte sedimentation rate (ESR) is a non-specific marker of inflammation. There is no adequate procedures for quality control monitoring because the ESR measure a physical phenomenon that depends on many variables. Therefore, our lab plan to establish control procedure using retained patient specimens.

Methods

We use Roller 20PN automated analyzers (Alifax, Italy) which capable of analyzing EDTA blood samples and reporting ESR result in 20 seconds.



Results

First, twenty-two specimens were selected with ESR range between 2 to 63 mm/hr, and then analyzed at 0, 2, 4, 8, 12, 24hrs after sampling(Fig. 1). The coefficient of determination and slope were calculated for each time-series. We expected that retained patient samples can be used as control materiel due to the ESR results remained stable within 24 hrs (r^2>0.94, slope 0.99-1.12, Table 1).

Furthermore, ninety-five were obtained and analyze ESR at 0, 24hrs. The quadratic regression curve between was calculated (y = -0.002x2 + 1.0617x -0.932, r2=0.9297, Fig. 2). Besides, we calculated the 95% confidence intervals for intercept, primary constant and secondary constant from the formula above mentioned(Table 2).

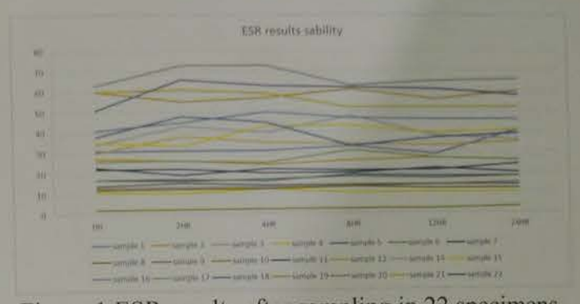


Figure 1 ESR results after sampling in 22 specimens

	0hr V.S. 2hr	0hr V.S. 4hr	0hr V.S. 8hr	0hr V.S. 12hr	0hr V.S. 24hr
r2	0.943	0.956	0.926	0.959	0.957
slope	1.120	1.118	1.020	0.999	1.010

Table 1 The slope and r2 between 0, 2, 4, 8, 12, 24hrs

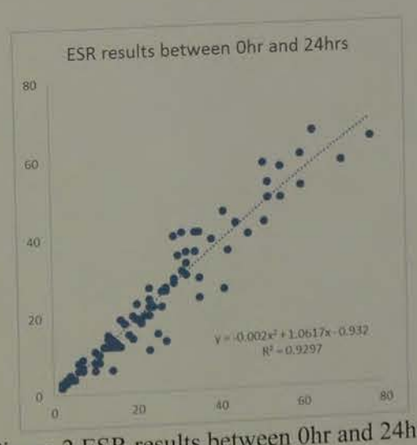


Figure 2 ESR results between 0hr and 24hrs

	Coefficient	Standard error	Lower limit of 95% confidence interval	Upper limit of 95% confidence interval
Intercept	-0.93204	1.12544	-3.16726	1.303176
Primary constant	1.061704	0.08478	0.893322	1.230085
Secondary constant	-0.002	0.001274	-0.00454	0.000526

Table 2 The 95% confidence intervals for intercept, primary constant and secondary constant

Conclusion

The limits of agreement for daily monitoring analytical system was based on calculation of the ESR result at 0 hour. In conclusion, we had established appropriate quality assurance procedure by using retained sample as quality control material.

Hematology

PC-07

Production of High Quality Specimen for Hematologic Diseases on Bone Marrow Examination

Sang-Muk Park

Dept. of Biomedical Laboratory Science, Dongkang College, Gwangju, 61200, Korea

Background : Recently the diagnosis of hematologic diseases could be made with peripheral blood due to cytogenetic chromosome analysis, immuno histochemistry, flow cytometry, FISH and development of molecular diagnostic test. But bone marrow examination is still compulsory for definite diagnosis of hematological diseases such as hematologic cancer and myeloproliferative neoplasms. Many medical institutions conduct bone marrow examination with medical technologists jointly, who must judge the appropriateness of bone marrow specimen harvested by doctors. Therefore, this study is to suggest for high quality bone marrow smear slide making methods, such as touch print and cytospin slide in cases of failed or clot of bone marrow aspiration.

Materials and methods : This study made success cases of bonemarrow biopsy in the adult patients having bone marrow examinationat hematology of C university hospital in Korea and bone marrow was harvested at the posterior superior iliac crest and used Jamshidi needle. Biopsy specimens touch print was made, and it was put in formalin solution of 10%, sent to the Department of Pathology, prepared with paraffin section and then estimation with Wright staining slide. When bone marrow aspiration specimen was coagulated and biopsy specimen was not suitable, we made cytospin slide using cytospin centrifuge and implemented Wright stain of the slide

Results : In case that bone marrow aspiration was failed, we implemented Wright stain of a well made touch print, and as a result of observation on microscope, we could be possible bone marrow cell differential count and estimation of cell morphology. When we did not have bone marrow aspiration, we made specimen due to dry tap etc., in case of bone marrow biopsy specimen, and implemented paraffin block using bone marrow biopsy specimen, and implemented Wright stain using the paraffin section. As a result of estimation of cell morphology using microscope, we accomplished effectively bone marrow cell differential count and estimation of cellularity. As a result that we calculated bone marrow cell differential count of cytospin slide, Wright stain was completed using microscope, the effective bone marrow cell differential count and estimation of cell morphology and for diagnosis, and report of the result was succeeded.

Hematology

PC-07

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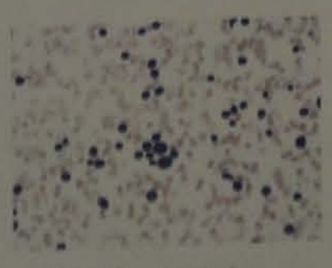
Touch print



TP Wright stain

Biopsy Wright stain

Cytospin slid WS

IFBLS, The 32nd World Congress of Biomedical Laboratory Science
August 31 (Wed.) - September 4 (Sun.) , 2016
Kobe international Conference Center , JAPAN

Hematology

PC-08

Myeloperoxidase Isozyme Expression in Cases of Chronic Myelogenous Leukemic Cells

Sang-Muk Park

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Background : Myeloperoxidase (MPO) is an antimicrobial enzymen the primary granule of neutrophils. Ion-exchange chromatography has determined that myeloperoxidase purified from human neutrophils takes three isozymal forms: MPO-I, MPO-II, and MPO-III. Wefound that two isozymes, designated MPO-1 and MPO-2, are expressed in crude MPO of neutrophils in normal persons

Materials and methods : In this study, we sought to determine whether the expression of MPO-1 and MPO-2 is related to prognosis in patients with chronic myelogenous leukemia (CML). Expression patterns were tested in the peripheral blood of three groups by means of native polyacrylamide gel electrophoresis (PAGE): normal persons (the normal group), patients with neutrophilia (the reactive group) as the control group, and patients with CML (the leukemic group).

Results : The expression of MPO-1 and MPO-2 in the leukemic group was more variable than in the normal group ($p<0.000$) but was similar to that in the reactive group. In addition to these isozymes, an abnormal band of MPO isozyme was found in 42% of the leukemic group but not in the other two groups.

Table 1. MPO isozymes in crude MPO of peripheral blood neutrophils from normal persons, reactive neutrophils from patients with neutrophilia and leukemic cell fro m patients with CML.

MPO-1	MPO-2	Number(%) Normal persons (A)	Patients with neutrophilia(B)	Patients with CML(C)	P-value A&B	A&C
+	+	25 (67.6 %)	15 (28.3%)	12 (38.8%)	0.000	0.000
+	-	12 (32.4 %)	15 (28.3%)	2 (6.4%)	0.000	0.000
-	+	0 (0.0 %)	1 (1.9%)	9 (29.0%)	0.000	0.000
-	-	0 (0.0 %)	22 (41.5%)	8 (25.8%)	0.000	0.000
		37 (100.0%)	53 (100.0%)	31 (100.0%)		

Chi-square test with exact test
MPO, myeloperoxidase; PAGE, polyacrylamide gel electrophoresis; CML, chronic myelocytic leukemia

Adjustments of cell concentrations from the collected neutrophils of normal persons using 10% native PAGE.

Table 2. The number of patient in which abnormal MPO was exhibited in crude MPO of neutrophils from normal persons, reactive neutrophils from patients with neutrophilia and leukemic cells from patients with CML.

	Exhibition pattern of abnormal MPO isozymes Normal persons		Patients with neutrophilia		Patients with CML		
(Number of patient)	(37)		(53)		(31)		
PB	-	+	-	+	-	-	+
BM	ND	ND	ND	ND	-	+	+
Number of patient	37	0	53	0	18	10	3
Total Number of patient which exhibit abnormal MPO (%)	0 (0.0%)		0 (0.0%)		13 (41.9%)		

+, Abnormal MPO isozyme is exhibited; -, Abnormal MPO isozyme is not exhibited; MPO, Myeloperoxidase; CML, Chronic myelocytic leukemia; ND, Not done; PB, Peripheral blood; BM, Bone marrow

Abnormal MPO isozyme of leukemic cells from 3 patients with CML using 10% native-PAGE.

Conclusion : The detection of this abnormal MPO isozyme on electrophoresis could be a useful indicator of CML. Further studies are needed to determine the sensitivity and specificity of this finding and whether it is a potentially acceptable clinical criterion for the diagnosis of CML. In addition, concurrent studies are in order to understand the molecular biology of this abnormal MPO isozyme.

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Hematology
PC-09

Hemostatic Potential of trunk extract of *Gliricidia sepium* (Jacq.) Kunth ex Walp.

Rexy Cordial Alvarez[1, 2] and Oliver Shane R. Dumaoal[2]
[1] Bicol Medical Center, Naga City, Camarines Sur, Philippines
[2] Lyceum of the Philippines University - Batangas Graduate School, Philippines

ABSTRACT

Plants used in traditional medicine provide an interesting and still largely unexplored source for the development of new drugs. *Gliricidia sepium* commonly known as Kakawate or Madre de Cacao is utilized for the management of coagulation disorders in traditional medical practice but there is no scientific study conducted yet to date that proves its hemostatic potential. This study investigated the hemostatic effect of trunk extract of *Gliricidia sepium*. Trunk extracts of *Gliricidia sepium* were obtained through squeezing process and tested for its hemostatic potential in citrated plasma through Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT) and Clotting Time (CT) using standard methods. Phytochemical screening of the trunk extract revealed the presence of sterols, alkaloids, saponins, and glycosides. The trunk extract significantly decreased both PT and APTT ($p<0.05$) with an insignificant increase in the CT ($p<0.05$). Results of the recent study indicated that there were significant differences on each concentration of treated samples in the PT and APTT when compared to the normal control. This was also observed when treated per concentration on a dose-dependent manner. The 4% concentration of trunk extract optimally decreased the PT ($p=0.0003$) while the 5.46% concentration of trunk extract optimally decreased the APTT ($p=0.0007$). This implies the hemostatic property of the trunk extract of *Gliricidia sepium* as well as its potential use and suitability for further development of the plant extract as a hemostatic agent.

METHODS

PHYTOCHEMICAL TESTING

COAGULATION TESTING

Sysmex

CA 500

RESULTS

Qualitative phytochemical analyses of trunk extract of *Gliricidia sepium*

Constituents	Result
Sterols	(+)
Triterpenes	(-)
Flavonoids	(-)
Alkaloids	(+)
Saponins	(+)
Glycosides	(+)
Tannins	(-)

Note: (+) Presence of constituents
(-) Absence of constituents

Effect of the trunk extract of *Gliricidia sepium* on PT

Group	Result (in seconds)
Normal Control	11.63±0.088
1800ul of blood	9.57±0.033
25 ul extract + 1800 ul of blood (1.377 %)	9.13±0.033
50 ul extract + 1800 ul of blood (2.7 %)	8.57±0.033
75 ul extract + 1800 ul of blood (4 %)	8.40±0.058
100 ul extract + 1800 ul of blood (5.26 %)	8.17±0.088

Data are expressed as mean ± SEM, $^{**}p<0.05$ compared to negative control group.

Effect of the trunk extract of *Gliricidia sepium* on APTT

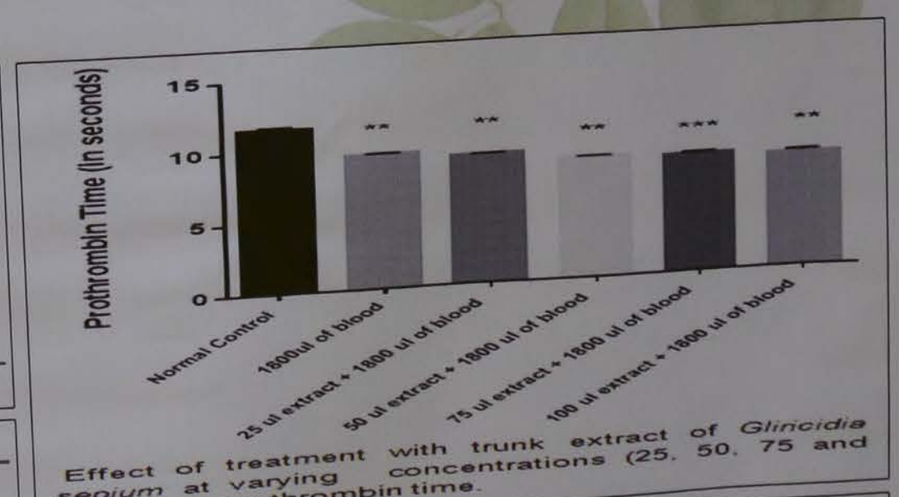
Groups	Result (in seconds)
Normal Control	37.03±0.088
1800ul of blood	36.17±0.088
25 ul extract + 1800 ul of blood (1.37%)	33.63±0.203
50 ul extract + 1800 ul of blood (2.7%)	29.80±0.153
75 ul extract + 1800 ul of blood (4%)	27.43±0.296
100 ul extract + 1800 ul of blood (5.26%)	26.60±0.153

Data are expressed as mean ± SEM, $^{**}p<0.05$ compared to negative control group

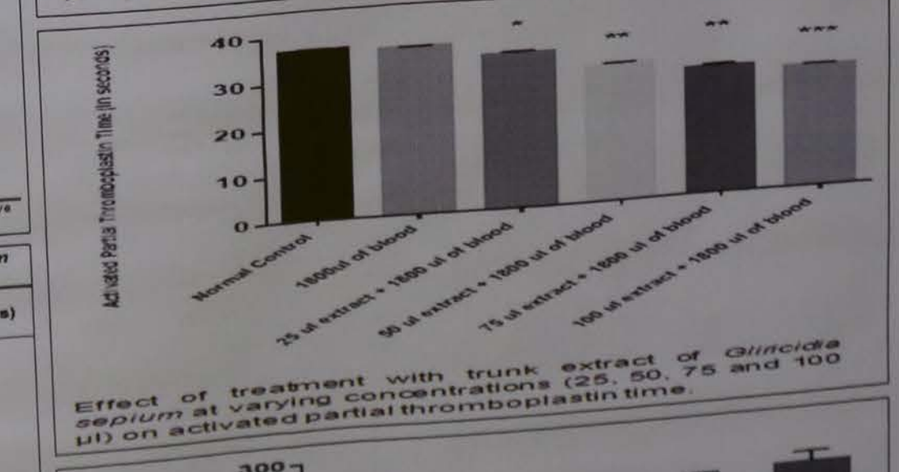
Effect of the trunk extract of *Gliricidia sepium* on Clotting Time

Group	Result (in seconds)
500 ul blood	248.3±10.14
25 ul extract + 500 ul of blood (4.7%)	192.7.3±3.71
50 ul extract + 500 ul of blood (9.0%)	210±5.77
75 ul extract + 500 ul of blood (13.0%)	228±6.01
100 ul extract + 500 ul of blood (16.0%)	241.7±10.14

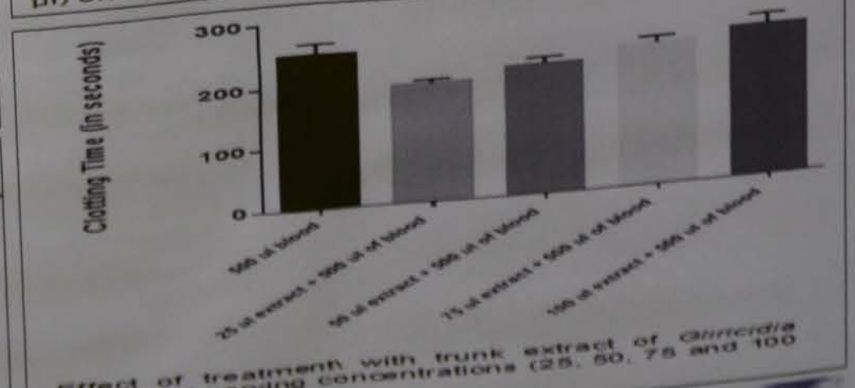
Data are expressed as mean ± SEM, $^{**}p<0.05$ compared to negative control group



Effect of treatment with trunk extract of *Gliricidia sepium* at varying concentrations (25, 50, 75 and 100 µl) on prothrombin time.



Effect of treatment with trunk extract of *Gliricidia sepium* at varying concentrations (25, 50, 75 and 100 µl) on activated partial thromboplastin time.



Effect of treatment with trunk extract of *Gliricidia sepium* at varying concentrations (25, 50, 75 and 100 µl) on clotting time.

CONCLUSION

The present study demonstrated that the trunk extract of *Gliricidia sepium* has significant procoagulant properties that can significantly decrease the PT and APTT. The result from (4%) concentration of trunk extract revealed the optimum concentration with significant decrease ($p=0.0003$) on PT while results of 5.46% concentration of trunk extract revealed the optimum concentration with significant decrease ($p=0.0007$) on APTT. The effects of the trunk extract on the said parameters are dose-dependent with increasing reduction in both PT and APTT in higher concentrations. Further in-depth studies on the trunk extract of *Gliricidia sepium* as an alternative procoagulant are recommended as well as fractionation of the plant constituents to determine the exact effect of each phytochemical contents.

Hematology

PC-10

PC-10

Development of an assay for activated platelet-derived microparticles by ELISA

Fundamental examination for combinations of platelet-related antibodies with sensitivity and specificity, reaction and conservation conditions, and instruments for sample collections.

Kazuki Shitamoto [1)], Kozue Okano, PhD [1)], Minako Araki [2)], Kaori Nakano [3)]
1)Faculty of Health Sciences, Yamaguchi University Graduate School of Medicine,
2)Onoda Red Cross Hospital , 3)Yamaguchi University Hospital

Introduction

Platelet-derived microparticle (PDMP) has a strong influence on the blood clot formation and inflammatory, however, measuring methods have not been established and mechanism of clot formation have not been clarified (Figure1). We conducted a fundamental examination of an assay by ELISA using various platelet-related antibodies to determine activated-PDMP (aPDMP). Additionally, we explored the relationship between various diseases under treatment and values of aPDMP in healthy volunteers and patients under optimal blood sampling and measuring conditions.

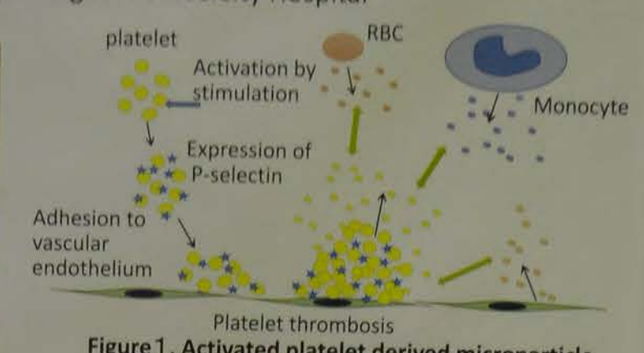


Figure 1. Activated platelet derived microparticle (aPDMP) and other cell-derived MPs

Materials and Method

○Calibration curve: Platelet rich plasmas (PRP) were prepared from healthy volunteers' citrated plasma. The platelet count of PRP was adjusted to 200,000/ul. Adjusted PRP were stimulated with 100μM ADP at 37℃ for 10 minutes, and sonicated to define aPDMP to obtain calibration curve.

○Blood sampling : We collected blood with 21G type needles into two vacuum collection tubes(1.8mL ・NIPRO)where citric acid was added, and only the second blood tubes were employed. Blood samples were centrifuged at 1200g for 10 minutes to extract supernatant.

○To confirm combination of various platelet-related antibodies for aPDMP: We used anti-vWF(DAKO)/anti-GP Ⅱb/Ⅲ/anti-AnnexinV (Miltenyi Biotec) antibodies for solid phases, and anti-GPIb (DAKOPATTS) /anti-CD40(CALTAG LABORATORIES)/anti-CD36 (IMMUNOTECH)antibodies for detectors and examined their sensitivity and specificity.

○Requirement study: Reaction temperatures were 4℃ to 37℃ and reaction times were one hour to overnight. Specificity was confirmed by the reactivity not only with platelet specimen but also with red blood cells and neutrophils.

○To clarify the optimal blood collecting device: 1) blood collection needle for vacuum tube (TERUMO), 2) flashback blood collection needle for vacuum tube (TERUMO), and 3) needle for syringe type (NIPRO), and compared the aPDMP values. In addition, we compared four kinds of vacuum blood collection tubes A(1.8mL,neotube,NIPRO,B(2.7mL,BDVacutainar,Becton,Dicknson),C(1.8 mL,Innsepac Ⅱ,SEKISUI),D(2.7mL,VENOJECT Ⅱ,TERUMO) which were commercially available and were by different makers.

○To investigate the relationship between disease treatment and aPDMP values: we measured aPDMP values of a total of 12 healthy volunteers(Age:22,female)and 121 patients (Age: 76±11.2, male: female=61: 60) with overlap diseases (4 with aneurysm, 75 on anticoagulant, 17 on drugs for ischemic heart disease, 8 on diuretic, 26 on drugs for health failure, and others).

Table 1. Evaluation of combinations of primary antibody and secondary antibody

Primary antibody		Secondary antibody		Peroxidase labeled antibody		evaluate	
						linearity	Concentration difference
AnnexinV monoclonal	×7500	vWF	×1000 ×5000 ×10000	rabbit	×500 ×1000 ×5000	○	×
		GPⅡb/Ⅲa (CD41/CD61)				×	×
AnnexinV Polyclonal	×1000	GPIb(CD42bc)	×1000 ×5000	mouse	×500 ×1000	◎	◎
		CD36				×	×
		CD40				×	×
		TP80(CDu41)				×	×
		P53				×	×
Von Willebrand Factor(vWF)	×1000	GPIb(CD42bc)	×1000 ×5000 ×10000	mouse	×1000	○	×
		CD36				○	×
		CD40				○	×
		TP80(CDu41)				×	×
		AnnexinV Mb	×5000			×	×
GPⅡb/Ⅲa (Integrinβ3)(CD41/CD61)	×1000	GPIb(CD42bc)	×5000	mouse	×500	×	×
		CD36				×	×
		AnnexinV Mb				×	×

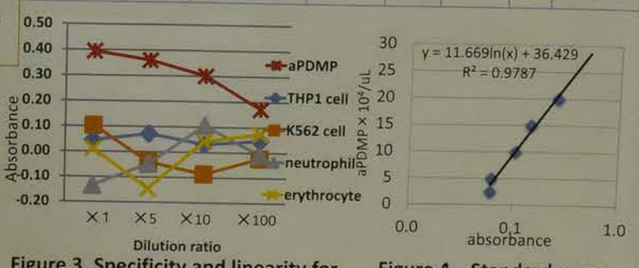


Figure 3. Specificity and linearity for aPDMP and other cell-derived MPs

Figure 4. Standard curve

Result

Table 2. Within-run reproducibility and daily precision

Within-run reproducibility			Daily precision (N=9)	
High (N=10)	mean	0.286	mean	23.5
	SD	0.0427		
	CV(%)	14.9	SD	2.967
Mid (N=10)	mean	0.0828		
	SD	0.0093	CV(%)	12.6
	CV(%)	11.2		
Low (N=10)	mean	0.0729		
	SD	0.0092		
	CV(%)	12.6		

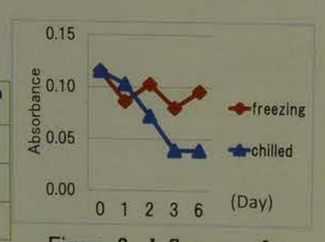


Figure 2. Influence of preservation methods

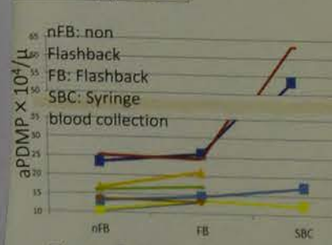


Figure 5. Influence of the blood collection needles

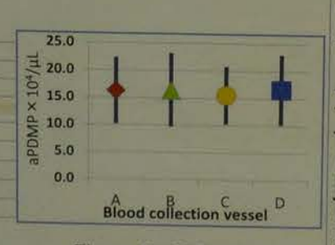


Figure 6. Differences in blood collection tubes

Table 3. Multiple regression analysis

	Factor	N	aPDMP	stdβ	P
	Healthy volunteer	12	14.70±5.38		
	Patient	121	13.03±7.31		
Multi-regression analysis in patients	Sex	121		0.0384	0.7031
	Age	121		0.0151	0.8755
	Aneurysm	4	5.90±2.30	-0.3238	0.0005
	Anticoagulant	75	11.61±6.69	-0.3301	0.0005
	Coronary heart disease medicine only	17	9.24±5.78	-0.3021	0.0016
	Heart failure	26	12.80±7.95	0.3501	0.0035
	Diuretic	8	9.47±3.73	-0.2529	0.0214
	Atrial fibrillation	49	11.14±5.74		N.S
	Diabetes mellitus	24	13.93±9.30		N.S
	Hyperlipidemia	12	13.91±10.27		N.S
	Arrhythmia	14	14.00±8.95		N.S

Result and Discussion

- The sensitivity and specificity of aPDMP varied according to the combination of platelet-related antibodies, combination of antiAnnexinV polyclonal antibody for solid-phase and anti-GPIb monoclonal antibody for detector was the most suitable for aPDMP (Table 1).
- For the optimal reactive conditions, temperature was 37℃ and reaction time was1 hour for solid-phase and detectors, and 4 hours for samples. Freezing was suitable to preserve samples (Figure 2). RBC, Neutrophil and Monocyte did not show non-specific response associated with this method (Figure 3). In the results, standard of aPDMP was linearity in a dose-dependent manner (Figure 4).
- The coefficient of variations showed within-run reproducibility of 11.2~14.9%, and daily precision of 12.6% (Table 2).
- The aPDMP values by different blood collecting devices were the lowest with the needle for vacuum tube and the highest with the needle for syringe type (Figure 5). There were no significant differences of the values among the four vacuum blood collection tubes by different makers (Figure 6).
- When the aPDMP values of healthy volunteers and patients were measured under the optimal conditions, the aPDMP values of patients were lower than those of healthy volunteers. It is suggested that majority of these patients are under treatment using one or more anticoagulant and antiplatelet agents and their aPDMP values were decreased compared to those of healthy subjects.
- Multi-regression analysis of aPDMP values in patients showed that the aPDMP values in patients with aneurysm, on anticoagulant, on drugs for ischemic heart disease, and on diuretic were significantly decreased, while those in patients with heart failure were significantly increased (Table 3).
- It is suggested that patients with heart failure produce significantly large amount of aPDMP or amount and types of treatment agents might not be appropriate. Further research with larger number of patients will be required in the future.

Hematology

PC-12

Case Report: A 9-yea… Chediak-Higashi Syn…

Chen Shuo-Hao[1] | Chen Mei-Zhi | L…
Taipei Tzu Chi Hospital, Buddhist Tzu Chi Medicine …

Background

Chediak-Higashi Syndrome (CHS) is … disorder that arises from a mutation of a ly… protein, which leads to a decrease in phag… exhibit hypopigmentation of the skin, eyes, …

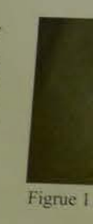
This disease damages immune system cel… fight off invaders such as viruses and bacter… with Chediak-Higashi Syndrome have repeat… starting in infancy or early childhood. These… serious or life-threatening.

Case Presentation

A 9-year-old female came to our hospital because of repeat fever. In the past, this patient also had history of regular visit the dermatologist because the skin spot lesions. When physical examination, she had pale face and her height is lower than other same age children and seems like a little sluggish in speech(Figure 1).

When analysis the CBC and differential count, we found there are some giant pink granules in neutrophils and lymphocytes cytoplasm. Combining her clinical symptoms and discussing with clinicians and pathologist, we concluded these granules are Chediak-Higashi anomaly (Figure 2,3,4).

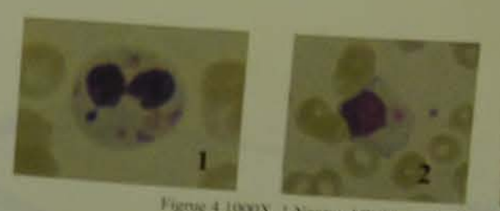
These granules are specific found in CHS patient's while blood cell cytoplasm. With the careful surveillance by the clinical laboratory scientist, found the characteristic white cells inclusions and discussion with clinicians, the patient had the right time of diagnosis and proper management.



Figrue 1

Figrue 2 400…

Figrue 3 1000X



Figrue 4 1000X, 1 Neutrophil, 2 Lymphcyte, 3 Eosinophil

Hematology

PC-11

The Effect of Specimen Hemolysis on PT and APTT test

Chen Mei-Chih[1] | Lin Yung-Shin | Chen Shuo-Hao | Wan Hsiang-Lin

Taipei Tzu Chi Hospital, Buddhist Tzu Chi Medicine Foundation, Department of Laboratory Medicine, New Taipei City Taiwan

Background

In the light of laboratory guideline, we reject PT and APTT samples when it appears hemolysis. But in our laboratory, we found there are no obvious differences between hemolysis or non-hemolysis sample had changed on final data.

Methods

A total of 159 patient samples with hemolysis and non- hemolysis were collected from the clinic laboratory. These samples tested PT, APTT or PT and APTT by clinicians' order. The hemolysis samples tested plasma hemoglobin level to identify the degree of hemolysis.

Results

There is no statistically significant differences between hemolysis or non-hemolysis samples in PT test ($r2=0.972$, $p>0.01$)(Figure 1), but a little differences in APTT test ($r2=0.8544$, $p>0.01$)(Figure 2). According to the CLIA' 88 guideline on test correlation limits, only one PT sample out of the 15% correlation limit (0.6%)(Figure 3), but there are 15 APTT samples out of the limit (9.4%)(Figure 4). There is no evidence show the relation between the data correlation and the degree of hemolysis(Figure 5,6).

Conclusion

These data show PT is more stable than APTT when in hemolysis sample. We recommend APTT should have a stricter rejected criterion than PT when sample has hemolysis.

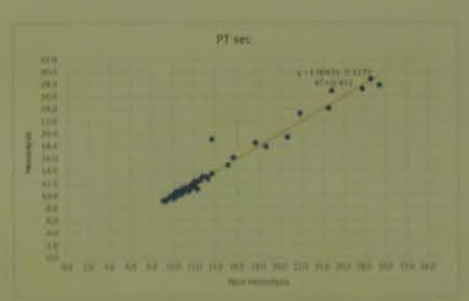


Figure 1 PT results between hemolysis and non-hemolisys

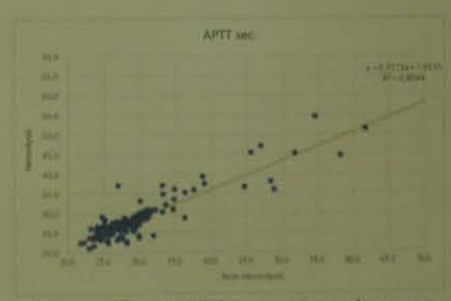


Figure 2 APTT results between hemolysis and non-hemolisys

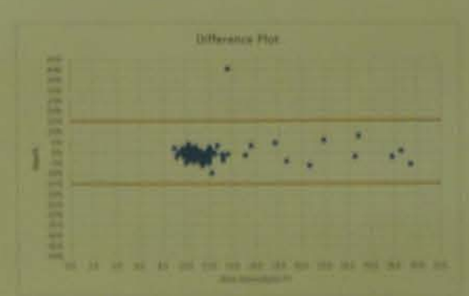


Figure 3 Difference Plot of PT bias(%) and non-hemolysis PT results

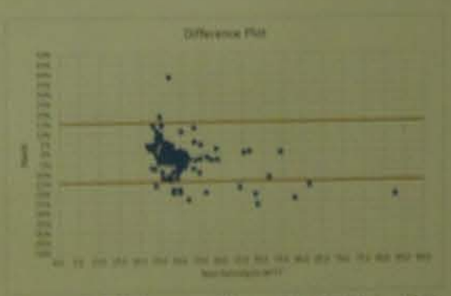


Figure 3 Difference Plot of APTT bias(%) and non-hemolysis APTT results

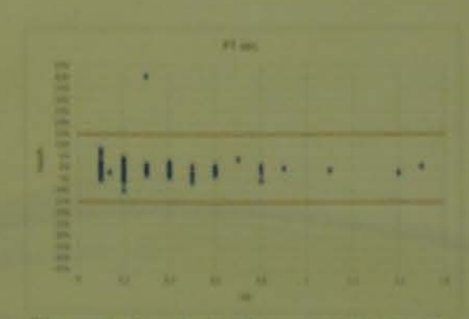


Figure 5 Correlation between PT bias(%) and Hb(g/dL)

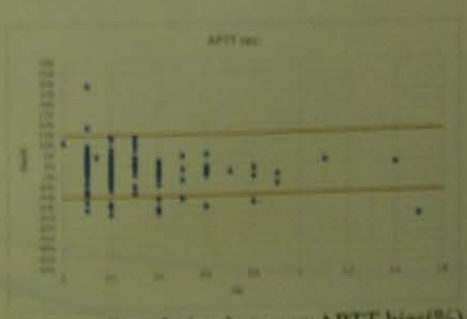


Figure 6 Correlation between APTT bias(%) and Hb(g/dL)

Hematology

PC-12

Case Report: A 9-year-old girl with Chediak-Higashi Syndrome

Chen Shuo-Hao | Chen Mei-Chih | Lin Yung-Shin | Wan Hsiang-Lin

Taipei Tzu Chi Hospital, Buddhist Tzu Chi Medicine Foundation, Department of Laboratory Medicine, New Taipei City Taiwan

Background

Chediak-Higashi Syndrome (CHS) is a rare autosomal recessive disorder that arises from a mutation of a lysosomal trafficking regulator protein, which leads to a decrease in phagocytosis. Patients with CHS exhibit hypopigmentation of the skin, eyes, and hair.

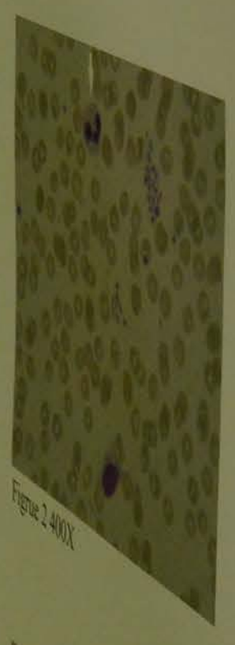
This disease damages immune system cells, leaving them less able to fight off invaders such as viruses and bacteria. As a result, most people with Chediak-Higashi Syndrome have repeated and persistent infections starting in infancy or early childhood. These infections tend to be very serious or life-threatening.

Case Presentation

A 9-year-old female came to our hospital because of repeat fever. In the past, this patient also had history of regular visit the dermatologist because the skin spot lesions. When physical examination, she had pale face and her height is lower than other same age children and seems like a little sluggish in speech(Figure 1).

Figrue 1 hypopigmentation of the skin

When analysis the CBC and differential count, we found there are some giant pink granules in neutrophils and lymphocytes cytoplasm. Combining her clinical symptoms and discussing with clinicians and pathologist, we confirmed these granules are Chediak-Higashi anomaly (Figure 2,3,4).



Figrue 2 400X

These granules are specific found in CHS patient's white blood cell cytoplasm. With the careful surveillance by the clinical laboratory scientist, found the characteristic white cell inclusions and discussion with clinicians, the patient had the right time of diagnosis and proper treatment.

Figrue 3 1000X

Figrue 4 1000X

Hematology

PC-12

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Chen Shuo-Hao[1] | Chen Mei-Zhi | Lin Yung-Shin | Wan Hsiang-Lin

Taipei Tzu Chi Hospital, Buddhist Tzu Chi Medicine Foundation, Department of Laboratory Medicine, New Taipei City Taiwan

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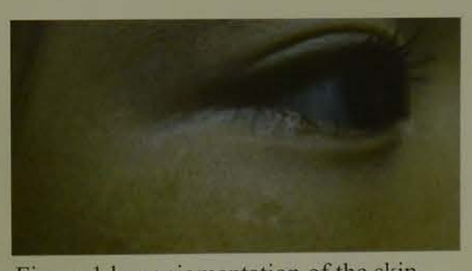

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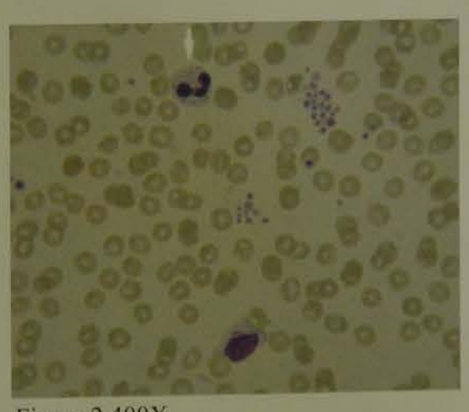

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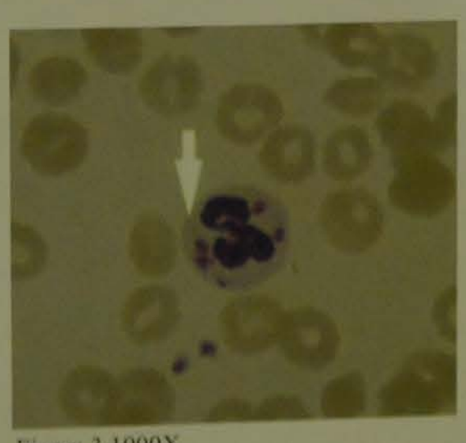

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佛教慈濟醫療財團法人 台北慈濟醫院
Taipei Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation

PC-11

The Effect of Specimen Hemolysis on PT and APTT test

Background

Methods

Results

Conclusion

Figure 1 PT results between hemolysis and non-hemolysis

Figure 2 Difference Plot of PT between hemolysis and non-hemolysis PT results

Figure 3 APTT results between hemolysis and non-hemolysis

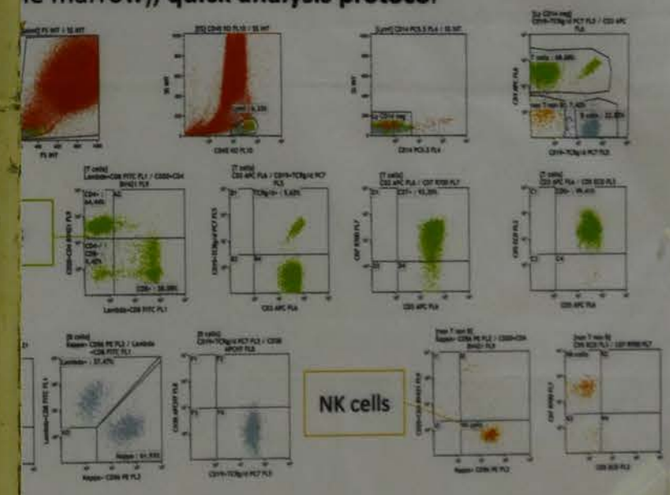
Figure 4 Difference Plot of APTT between hemolysis and non-hemolysis APTT results

FLOW CYTOMETRIC LYMPHOCYTE SCREENING WITH 14 ANTIBODIES IN 10 COLOURS

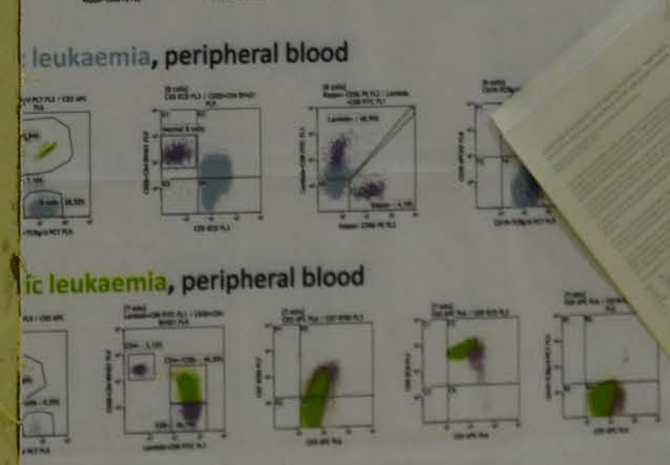
Kiitta Kemppi and Arja Alkula, Fimlab Laboratories, Tampere, Finland

	PC5.5	PC7	APC	APC-R700	APC-H7	BV421	Krome Orange
	CD14	CD19 TCRγδ	CD3	CD7	CD38	CD20 CD4	CD45

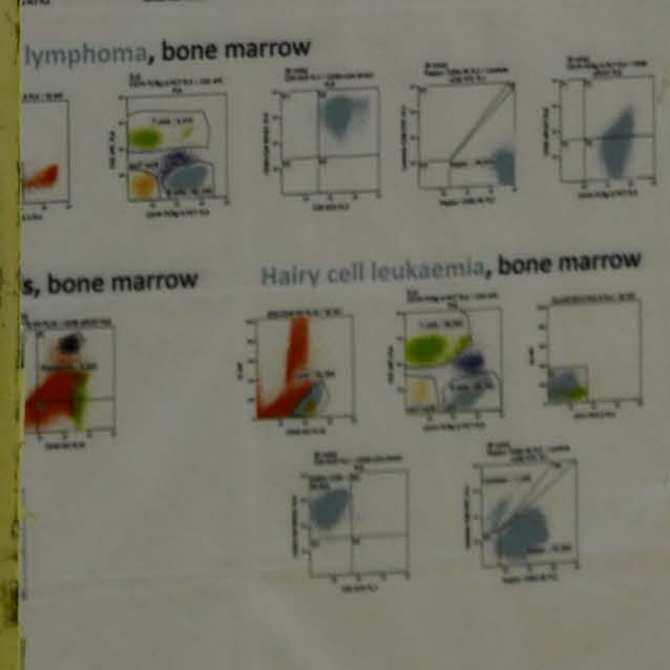
ne marrow), quick analysis protocol



NK cells



leukaemia, peripheral blood



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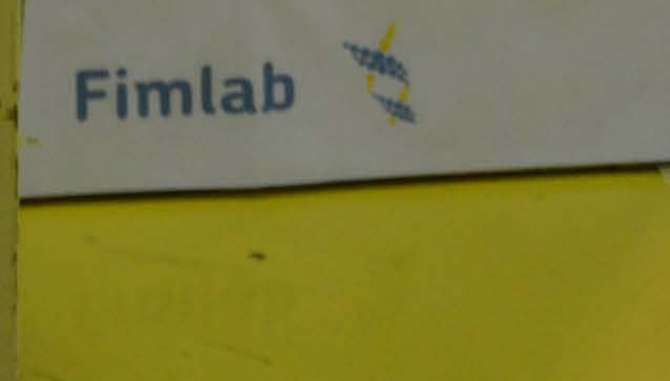

lymphoma, bone marrow

s, bone marrow

Hairy cell leukaemia, bone marrow

Fimlab

Hematology

PC-13

Research and development of portable device for hemoglobin concentration test
- The test performance for the Point of Care Testing -

H Nozaka[1)] , M Ooura[2,3)], A Megumi[1)], H Sazawa[1)], M Fujioka[1)], H Takami[1)], K Kida[1)]
1) Graduate school of health sciences, Hirosaki university 2) Graduate school of humanities and social sciences, Hirosaki university 3) CONSIS, INC

台北慈濟醫院
Taipei Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation

The Effect of Specimen Hemolysis on PT and APTT test

Chen Mei-Chih | Lin Yung-Shin | Chen Shuo-Hao | Wan Hsiang-Lin
Taipei Tzu Chi Hospital, Buddhist Tzu Chi Medicine Foundation, Department of Laboratory Medicine, New Taipei C

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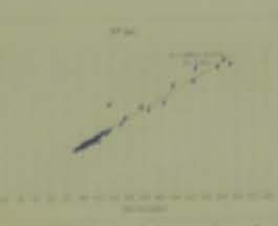


re 1 PT results between hemolysis non-hemolisys

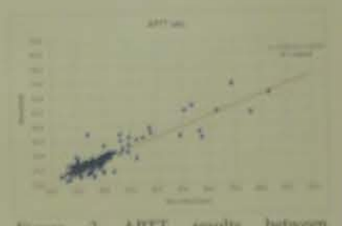
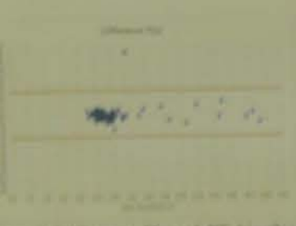
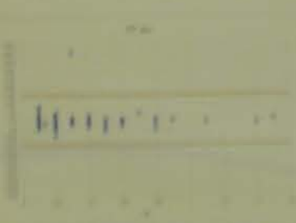


Figure 2 APTT results between hemolysis and non-hemolisys



ure 3 Difference Plot of PT bias(%) non-hemolysis PT results

Figure 3 Difference Plot of APTT bias(%) and non-hemolysis APTT results



gure 5 Correlation between PT bias(%) d Hb(g/dL)

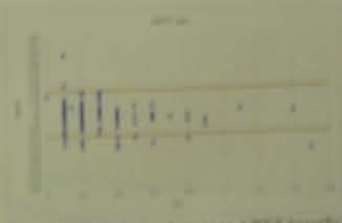


Figure 6 Correlation between APTT bias(%) and Hb(g/dL)

Research and development of portable device for hemoglobin concentration test
- The test performance for the Point of Care Testing -

H Sazawa[1)], M Aita[1)], H Nozaka[1)] , M Ooura[2,3)], M Fujioka[1)], H Takami[1)], K Kida[1)]
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HIROSAKI UNIVERSITY

Background

1 **Transition from Hospital to Home therapy**
It is required that chronic phase medical care is converted into home therapy, because lack of medical resources has caused in Japan.

2 **Nutritional management**
Hemoglobin level testing which is significant marker of anemia. It is important for health management in home nursing and NST activities.

Self-medication
POCT devices that nurses or public health nurses are able to operate easily in the home health nursing is very few.

Objectives

The aim of this study is development of POCT device for health management in home therapy.
The condition of this system is following

1. Minimally invasive and blood volume
2. Low hardware price and running cost
3. Maintenance free
4. High accuracy compare with Lab test

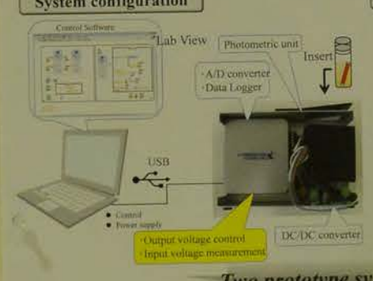
Auto analyzer vs POCT devices (Our target)

	Automatic blood cell analyzer	Hb analyzer (POCT device)	Our POCT device
The minimum blood volume	300~400μL	10μL	1~2μL
Accuracy	◎	○	○
Precision	◎	○	○
Measuring time	1 min	1min	Less than 1min
Device price	50000~100000 dollars	2000 dollars	300 dollars
Portability	×	350g	Less than 500g

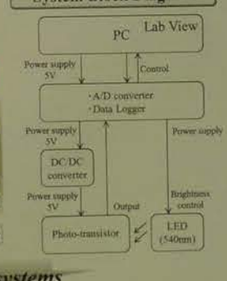
System configuration

The portable device was consisted of photo-transistor unit, LED unit, and data logger system "NI-USB DAQ". Application software was developed by "NI-LabVIEW". Hemoglobin test principle was used SLS-hemoglobin method.

System configuration



System Block Diagram



Two prototype systems

Type A : Hb self mini α — Optical path length 10mm

Type B : Hb self mini β — Optical path length 5mm

Hb test process

Conclusion

- Our system needs small amount of blood collection, and operability is also simple and easy.
- The drawing by the lancet is low-invasive, and not only nurse but also patient are able to drew blood easily. But this method tends to cause an error, it seems that patients need do training before Hb tests.
- It seems that our system is useful for medical activities in out-of-hospital, but it is required to evaluate patients blood with anemia in the clinical field.

Utilization field

System evaluation

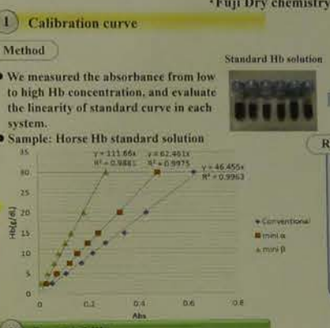
Evaluation method
- Calibration curve
- Repeatability
- Correlativity

Compare Devices
- Spectrophotometer (Conventional)
- Hb self mini α
- Hb self mini β
- Fuji Dry chemistry 4000

SLS method

1 Calibration curve

Method
- We measured the absorbance from low to high Hb concentration, and evaluate the linearity of standard curve in each system.
- Sample: Horse Hb standard solution

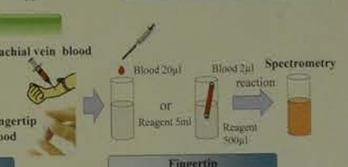


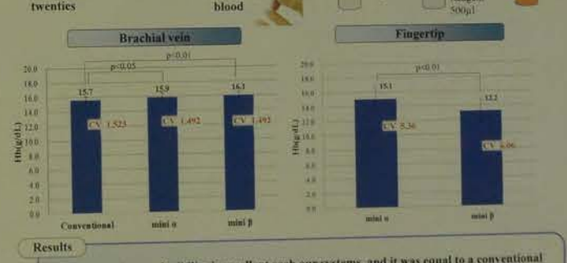
Results
- The linearity of each system is excellent from 2.5g/dL to 30g/dL.
- Our systems were low resolution compared to the conventional method

A necessary and sufficient condition was satisfied.

2 Repeatability

Method
- We measured repeatedly 10 times with peripheral blood from brachial vein and fingertip blood.
- Samples: Healthy person in twenties



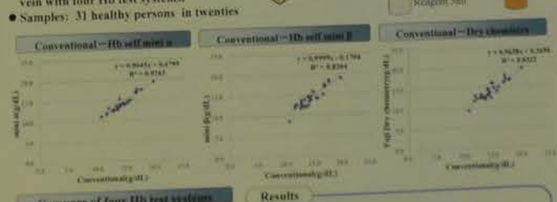


Results
- The repeat reproducibility is excellent each our systems, and it was equal to a conventional method with peripheral blood from brachial vein .
- The repeat reproducibility is poor each our systems with fingertip blood by lancet.

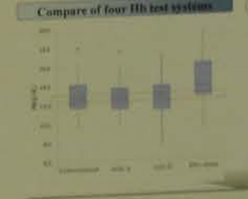
The repeat reproducibility was satisfied in our system.
It is suspected that tissue fluid is mixed in blood by Lancet drawing.

3 Correlativity

Method
- We measured peripheral blood from brachial vein with four Hb test systems.
- Samples: 31 healthy persons in twenties



Compare of four Hb test systems



Results
- Mini α system showed good correlation with conventional system.
- Mini β system showed a tendency of low value.
- Fuji Dry chemistry showed a tendency of high value.

The correlativity was excellent in our system.

Hb self mini satisfies necessary and sufficient condition for POCT.

Acknowledgement and COI

Acknowledgement
This work was supported by SCOPE of the Japan Ministry of Internal Affairs and Communications.

Conflict of interest
The authors have no conflict of interest (COI) directly relevant to the content of this article

Hematology

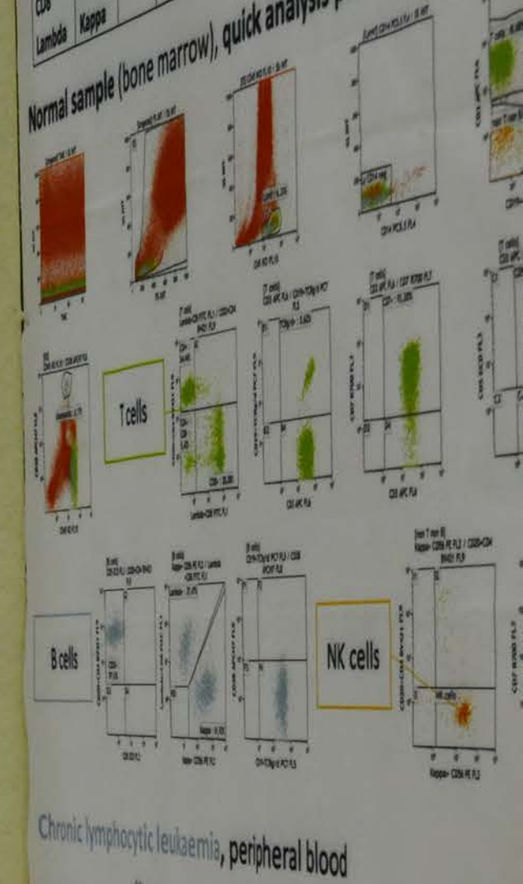
PC-14

FLOW CYTOMETRIC LYMPHOC SCREENING WITH 14 ANTIBO IN 10 COLOURS

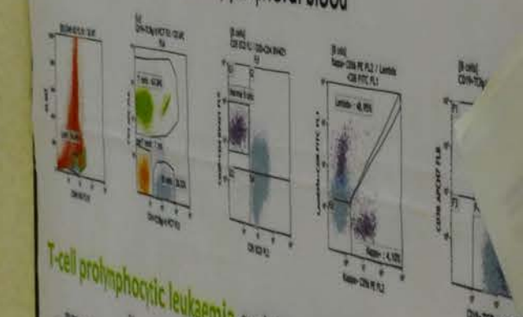
Riitta Kemppi and Arja Aikula, Fimlab Labora
Tampere, Finland

		APC	APC-R700	APC-H7		B/421		
	PC7				CD38		CD20	
FITC	PE	ECD	PC5.5	PC7		CD7		CD4
CD8	CD56	CD5	CD14	CD19	CD3			
Lambda	Kappa			TCRγδ				

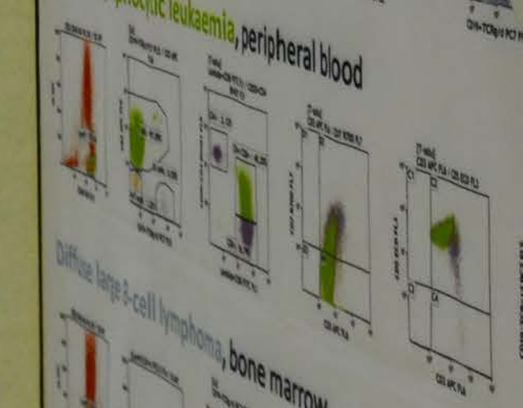
Normal sample (bone marrow), quick analysis protocol



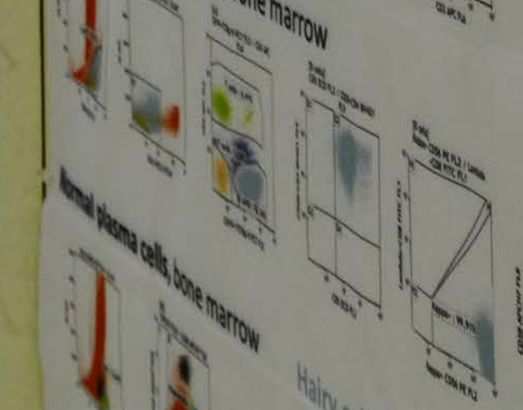
Chronic lymphocytic leukaemia, peripheral blood



T-cell prolymphocytic leukaemia, peripheral blood

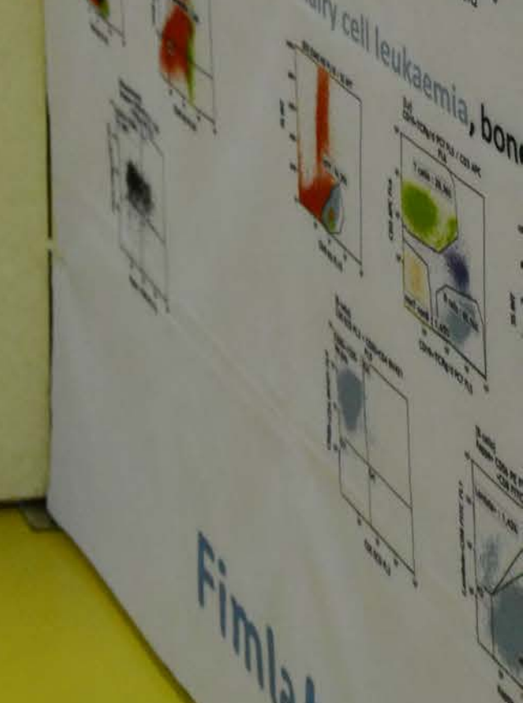


Diffuse large B-cell lymphoma, bone marrow



Normal plasma cells, bone marrow

Hairy cell leukaemia, bone



Fimlab

Hematology

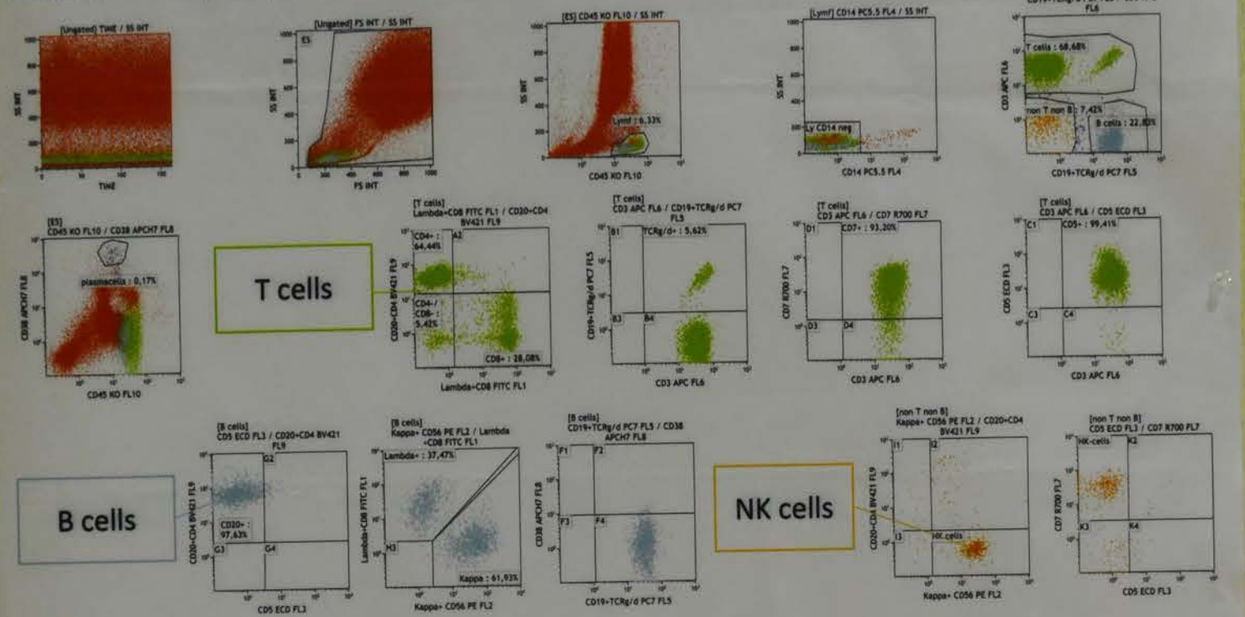
PC-14

FLOW CYTOMETRIC LYMPHOCYTE SCREENING WITH 14 ANTIBODIES IN 10 COLOURS

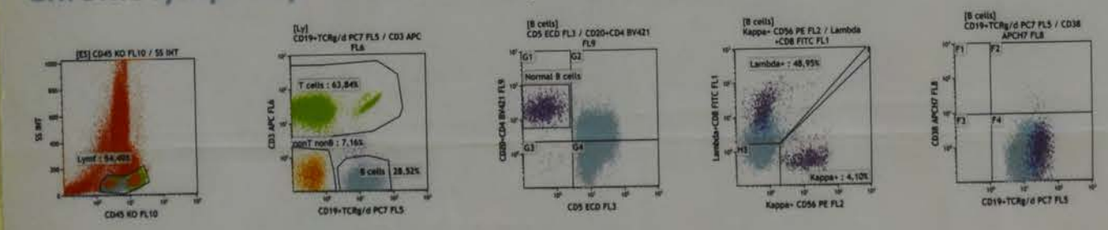
Riitta Kemppi and Arja Alkula, Fimlab Laboratories, Tampere, Finland

FITC	PE	ECD	PC5.5	PC7	APC	APC-R700	APC-H7	BV421	Krome Orange
CD8 Lambda	CD56 Kappa	CD5	CD14	CD19 TCRγδ	CD3	CD7	CD38	CD20 CD4	CD45

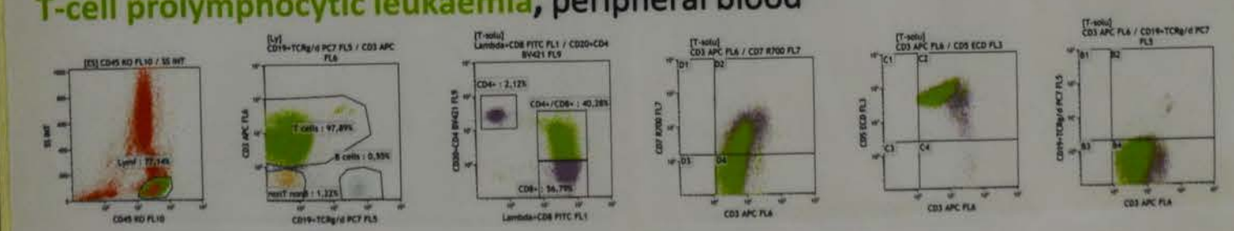
Normal sample (bone marrow), quick analysis protocol



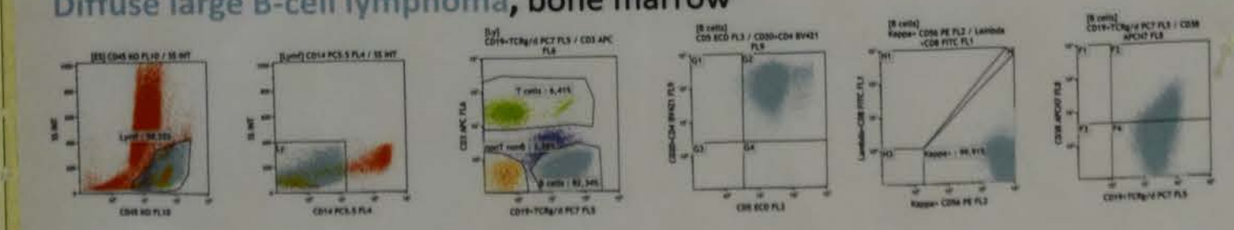
Chronic lymphocytic leukaemia, peripheral blood



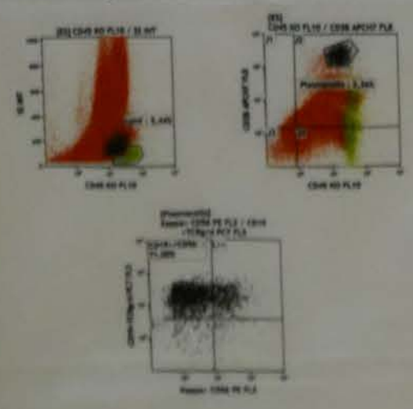
T-cell prolymphocytic leukaemia, peripheral blood



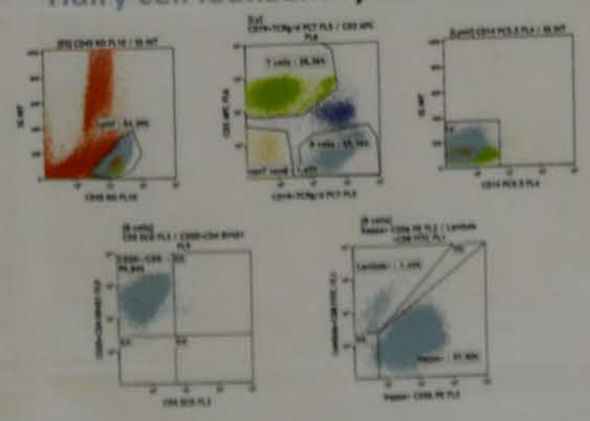
Diffuse large B-cell lymphoma, bone marrow



Normal plasma cells, bone marrow



Hairy cell leukaemia, bone marrow



Fimlab

Hematology
PC-13

Research and development of portable device for hemoglobin concentration test
- The test performance for the Point of Care Testing -

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Research and development of portable device for hemoglobin concentration test
- The test performance for the Point of Care Testing -
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HIROSAKI UNIVERSITY

Background

1 Transition from Hospital to Home therapy
It is required that chronic phase medical care is converted into home therapy, because lack of medical resources has caused in Japan.
Medical needs — Low-invasive, Low cost, Easy — New POCT device for home nursing — Chronic Lab data in home

2 Nutritional management
Hemoglobin level testing which is significant marker of anemia. It is important for health management in home nursing and NST activities.

Self-medication
Problem
POCT devices that nurses or public health nurses are able to operate easily in the home health nursing is very few.

Objectives

The aim of this study is development of POCT device for health management in home therapy.
The condition of this system is following

- Minimally invasive and blood volume
- Low hardware price and running cost
- Maintenance free
- High accuracy compare with Lab test

Auto analyzer vs POCT devices — Our target

System configuration

The portable device was consisted of photo-transistor unit, LED unit, and data logger system "NI-USB DAQ". Application software was developed by "NI-LabVIEW". Hemoglobin test principle was used SLS-hemoglobin method.

System configuration — System Block Diagram

Two prototype systems

Type A : Hb self mini α — Optical path length 10mm
Type B : Hb self mini β — Optical path length 1mm

Hb test process

STEP1 Initialization — STEP2 Cmc blood sampling — STEP3 Measurement

Conclusion

- Our system needs small amount of blood collection, and operability is also simple and easy.
- The drawing by the lancet is low-invasive, and not only nurse but also patient are able to draw blood easily. But this method tends to cause an error, it seems that patients need do training before Hb tests.
- It seems that our system is useful for medical activities in out-of-hospital, but it is required to evaluate patients blood with anemia in the clinical field.

Utilization field

System evaluation

Evaluation method — Compare Devices
- Calibration curve
- Repeatability
- Correlativity

- Spectrophotometer (Conventional)
- Hb self mini α
- Hb self mini β
- Fuji Dry chemistry 4000

SLS method

1 Calibration curve
Method
- We measured the absorbance from low to high Hb concentration, and evaluate the linearity of standard curve in each system.
- Sample: Horse Hb standard solution

Standard Hb solution — Standard 20μl — Spectrometry — Reagent 5ml

Results
- The linearity of each system is excellent from 2.5g/dL to 30g/dL.
- Our systems were low resolution compared to the conventional method

A necessary and sufficient condition was satisfied.

2 Repeatability
Method
- We measured repeatedly 10 times with peripheral blood from brachial vein and fingertip blood.
- Samples: Healthy person in twenties

Brachial vein blood — Fingertip blood — Blood 20μl — Blood 2μl — Reagent 500μl — Spectrometry

Brachial vein — Fingertip

Results
- The repeat reproducibility is excellent each our systems, and it was equal to a conventional method with peripheral blood from brachial vein.
- The repeat reproducibility is poor each our systems with fingertip blood by lancet.

The repeat reproducibility was satisfied in our system.
It is suspected that tissue fluid is mixed in blood by Lancet drawing.

3 Correlativity
Method
- We measured peripheral blood from brachial vein with four Hb test systems.
- Samples: 11 healthy persons in twenties

Brachial vein blood

Results
- Mini α system showed good correlation with conventional system.
- Mini β system showed a tendency of low value.
- Fuji Dry chemistry showed a tendency of high value.

The correlativity was excellent in our system.

Hb self mini satisfies necessary and sufficient condition for POCT.

Our hand out Take Free
ご自由にお取りください

Acknowledgement and COI

Acknowledgement
This work was supported by SCOPE of the Japan Ministry of Internal Affairs and Communications.

Conflict of interest
The authors have no conflict of interest (COI) directly relevant to the content of this article.

Hematology
PC-15

Discrepancy between conventional cytogenetic analysis and FISH signals for *RUNX1/RUNX1T1*

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Hematology
PC-16

Background

Translocation (8;21)(q22;q22) is one of the most recurrent cytogenetic abnormalities in acute myeloid leukemia (AML). Typically, the *RUNX1/RUNX1T1* rearrangement shows balanced translocation between chromosomes 8 and 21. This case report is about variant forms of translocation caused by cryptic insertion of *RUNX1T1* (8q22) gene on *RUNX1* (21q22) gene. These forms of translocation are hardly detectable by conventional cytogenetic studies. Fluorescence in situ hybridization (FISH) or reverse transcriptase polymerase chain reaction (RT-PCR) techniques are needed to reveal the rearrangements.

Fig 3. Metaphase FISH using *RUNX1/RUNX1T1* dual-color dual fusion probe (Vysis)

Case Report

A 47 year old woman referred to our hospital was diagnosed with AML based on morphologic, cytochemical, and immunophenotypic findings on bone marrow studies. Peripheral blood counts were: hemoglobin 9.7 g/dL, platelets 32×10³/μL, and leukocytes 9.0/μL with 3% myelocytes, 26% neutrophils, 20% lymphocytes, 3% monocytes, 1% eosinophils, and 47% immature cells. Bone marrow was hypercellular marrow(71~80%), blast cells counted up to 43.5% and were strongly positive for peroxidase and nonspecific esterase reaction, negative for P.A.S. The blast cells expressed CD13, CD19, CD33, CD34, CD56, Cyt CD79a, Cyt IgM, CD117 and MPO. Conventional cytogenetic analysis revealed 45,X,-X[18]/46,XX[2]. But, interphase fluorescence in situ hybridization (FISH) for the *RUNX1/RUNX1T1* rearrangement showed chimeric fusion gene in 96% of bone marrow nucleated cells, one fusion, two orange, one green signal pattern(1F2O1G) using *RUNX1/RUNX1T1* dual-color dual fusion probe[Vysis; Abbott Molecular, Downers Grove, IL, USA, *RUNX1T1*(orange), *RUNX1*(green)]. Meanwhile, typical pattern of *RUNX1/RUNX1T1* rearrangement is two fusion, one orange and one green signal. In this patient, metaphase FISH revealed that one fusion signal was located on the long arm of chromosome 21, but no fusion signal on derivative chromosome 8. Additionally, *RUNX1/RUNX1T1* rearrangement was accompanied by the loss of a whole X chromosome which is the most common additional cytogenetic aberration in AML with *RUNX1/RUNX1T1*.

Fig 4. Inverted-DAPI and merged color of Fig 3.

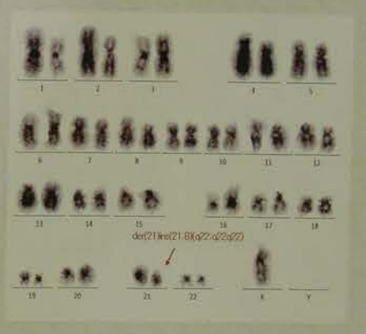

Fig 5. Karyotyping on Inverted grey scale.

Molecular genetic study by reverse transcriptase polymerase chain reaction (RT-PCR) confirmed the presence of the chimeric transcript for *RUNX1/RUNX1T1*. The final karyotype was 45,X,-X,ish ins(21;8)(q22;q22q22)(*RUNX1T1*+;*RUNX1*+,*RUNX1T1*+)[18]/46,XX[2]

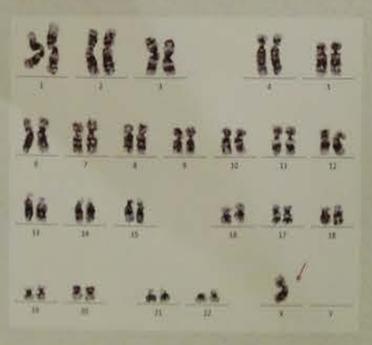

Fig 1. G-banded karyotype of BM
45,X,-X[18]/46,XX[2]

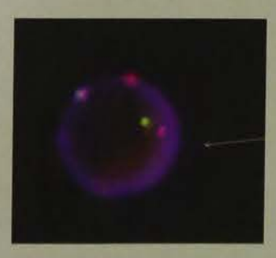

Fig 2. *RUNX1/RUNX1T1* dual-color dual fusion probe (Vysis; Abbott Molecular, Downers Grove, IL, USA)
RUNX1T1(orange), *RUNX1*(green) one fusion, two orange, one green signal

Discussion

We experienced the cryptic rearrangement that is hardly detected by conventional cytogenetic analysis. Molecular genetic studies such as FISH or RT-PCR is very useful tool to detect the masked type of cryptic translocation.

References

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Frank G. Rücker,Lars Bullinger,Alexander Gribov,Martin Sill,Richard F. Schlenk,Peter Lichter,Hartmut Döhner,Konstanze Döhner. Molecular characterization of AML with ins(21;8)(q22;q22q22) reveals similarity to t(8;21) AML

Hematology
PC-17

Clonality study of T-cell Lymphoproliferations in Taiwan

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AIMS

Malignant lymphoma is a clonal proliferation of a malignantly transformed lymphocyte of B-cell, T-cell, or natural killer (NK) cell. The clonal nature of lymphoma cells can be elucidated by B-cell immunoglobulin gene or T-cell receptor gene rearrangement study. Distinguishing malignant lymphoma from a reactive process is sometimes a big challenge and this is the major reason that molecular diagnostic assays for clonality have become increasingly important and popular in the clinical diagnostic laboratories. These assays are performed to determine the clonality of suspected lymphoproliferations. In our previous study we have successfully used polymerase chain reaction (PCR) method and conventional primers to detect the clonal gene rearrangements of immunoglobulin heavy chain gene (IGH) and T-cell receptor γ chain gene (TCRG) for B-cell and T-cell clonality, respectively. A big study from 45 top European laboratories led to the launch of BIOMED-2 multiplex primers in 2003.

METHODS

We retrospectively searched for T-cell lymphoma in our institute from 2001 to 2015 and identified 40 cases. PCR-based analysis was performed on all samples from peripheral blood (PB) or bone marrow (BM) aspirate and paraffin-embedded tissues.

RESULTS

We compared the conventional and BIOMED-2 primers and found that the latter was more sensitive. The most efficient and cost-effective way to detect clonal TCR gene rearrangement (TCR-GR) was using the in-house TCRG primers as the initial step. The polyclonal cases would be subject to the two BIOMED-2 TCRG reactions. With this strategy, 62.5% of T-lineage neoplasms in this study were clonal at the initial screening and an additional 27.5% would be clonal by the two BIOMED-2 TCRG reactions. By combining these four reactions we could detect clonal TCR-GR in 90% cases, higher than either in-house TCRG alone or all six BIOMED-2 TCR reactions alone.

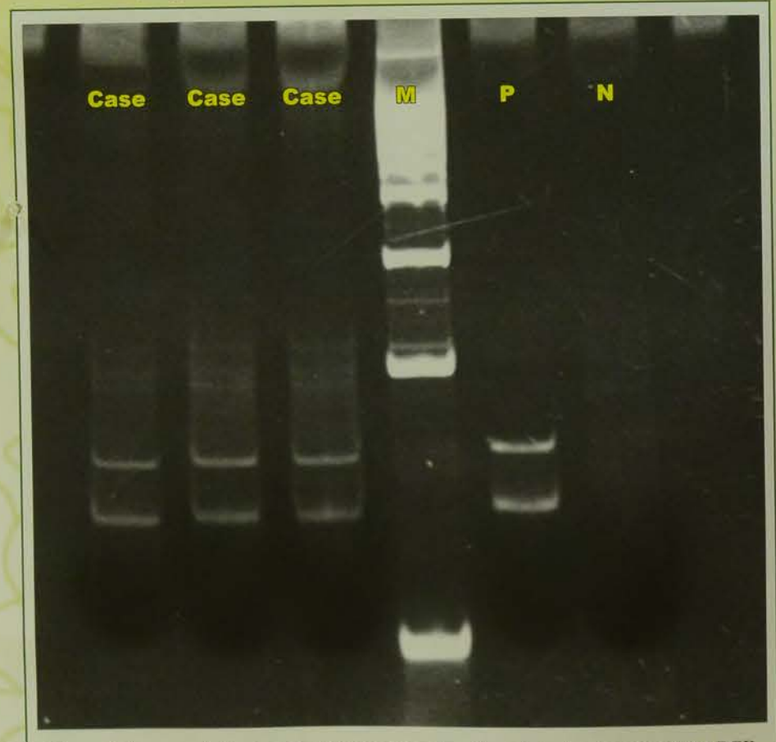

Figure 1 Representative images form polyacrylamide gel electrophoresis of the PCR products

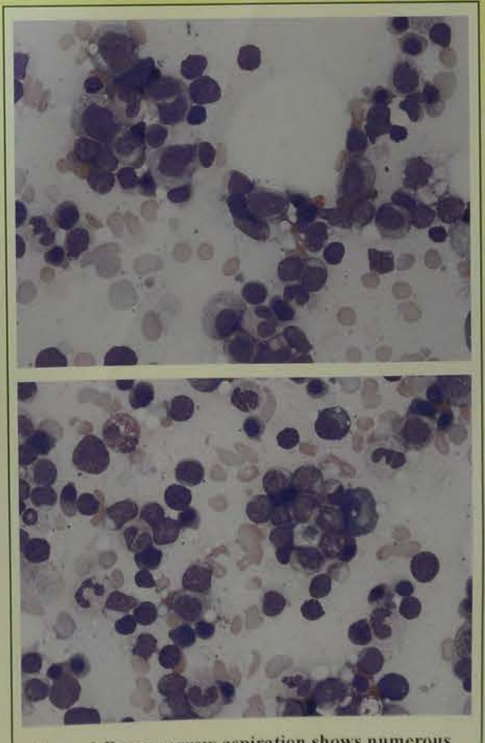

Figure 2 Bone marrow aspiration shows numerous abnormal lymphocytes with irregular nuclear contous

DISCUSSION

Due to various factors including limited National Health Insurance coverage of molecular testing, clonality study for lymphoproliferations was rarely performed for routine diagnosis in Taiwan until recently. We have previously used the same inhouse

TCRG primers for a smaller series of T-cell lymphomas and detected clonal TCR-GR in 75% of cases. From our case series, the combined use of two in-house TCRG

tubes and two BIOMED-2 TCRG tubes, that is, the four-tube strategy, will detect a clonal TCR gene rearrangement in 90% of cases with a confirmed diagnosis of T-cell neoplasm with adequate DNA quality. Performing other BIOMED-2 reactions is

needed only for the remaining of cases, of which 3% are likely to show a clonal TCRB and/or TCRD gene rearrangement. Such an overall level of T-cell clonality detection rate compares well with the 94% detection rate reported in the follow-up study

of the BIOMED-2 group and the 93-96% detection rate reported in the independent studies using BIOMED-2 assays combined with laboratory developed assays on fresh-frozen materials. Thus, approximately 5% of false negative results are expected in T-cell malignancies with adequate DNA quality. The causes for the false negative results are unclear. Incomplete coverage of the rearranged TCR gene segments by the primers designed or unusual rearrangement of TCR genes may be a factor.

In summary, we found from this parallel study that PCR-based clonality assessment using in-house TCRG primers was an adjunct to the BIOMED-2 protocols for detecting TCR-GR. By combining the in-house and BIOMED-2 TCRG reactions with a total of four tubes, we could achieve a relatively high detection rate (90%) at a relatively low cost. As the in-house primers could be custom made and are much cheaper than the commercial BIOMED-2 kits, we concluded that this four-tube strategy was cost-effective and efficient for TCR clonality assessment.

Hematology

PC-18

Ethnic Difference of Chromosome Aberrations and Genetic Mutations in Chronic Lymphocytic Leukemia

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Introduction

Chronic lymphocytic leukemia (CLL) is considered as typical malignancy with ethnic difference, which is one of the common leukemia in western countries, while extremely rare in Asian countries[1,2]. To investigate whether chromosome aberration and genetic mutation of Korean CLL patients is different from that of Caucasian, we performed conventional cytogenetic test, fluorescent in situ hybridization (FISH) and target capture sequencing for 87 hematologic malignancy related genes using custom designed capture panel.

Materials and Methods

Study Population

A total of 71 patients diagnosed with CLL/SLL between September 2001 and October 2013 at Seoul National University Hospital (SNUH, n=58) and Asan Medical Center (n=13) were enrolled. All of the patients were Korean.

Methods

▪G-banding and Fluorescence *in situ* hybridization

-For G-banding, B cell-mitogen, tetradecanoylphorbol acetate (TPA; phorbol-12-myristate-13-acetate) was added with subsequent culture for 4 days.

-FISH for enumeration of chromosome 12 and for detection of 13q14.3 deletion, 17p13 deletion, 11q22 deletion and *IgH/CCND1* translocations (to exclude mantle cell lymphoma) was performed: the LSI D13S319/LSI13q34/CEP12 Multi-color Probe, LSI *TP53* (17p13.1) SpectrumOrange Probe, Vysis *IGH/CCND1* XT DF FISH Probe (all from Abbott Molecular/Vysis, Des Plaines, IL, USA), and XL *ATM/TP53* Probe (Metasystems, GmbH, Altlussheim, Germany).

▪Targeted sequencing

-To gain insight into the genetic lesions that drive CLL, we performed targeted sequencing for 87 hematology malignancy-related genes . gDNA shearing to generate the standard library and the hybridization step targeting only exonic regions were performed by Celemics Inc. (Seoul, Korea).

-The final quality was assessed using the Agilent 2200 TapeStation System (Santa Clara, CA, USA).

-We sequenced a total target length of 259-kb regions using the paired-end 150-bp rapid-run sequencing mode on an Illumina HiSeq 2500 platform. The mean sequencing depth for the targeted regions (259 kb) was 231-fold (n=48).

Results

Conventional karyotyping and fluorescence *in situ* hybridization

Cytogenetic abnormalities were examined through G-banding (60/71 patients) and FISH studies (51/71 patients). Aberrant karyotypes were observed in 28.3% of patients by G-banding and 66.7% of patients by FISH. Deletion of 13q14 was detected in 45.8% (22/48 patients), followed by trisomy 12 in 30.0% (15/50 patients), deletion of 17p13 in 23.5% (12/51 patients), and deletion of 11q22 in 15.9% (7/44 patients).(Table 1, Table2)

Table 1. Cytogenetic aberrations detected by G-banding and FISH studies in 60 Korean Patients with CLL

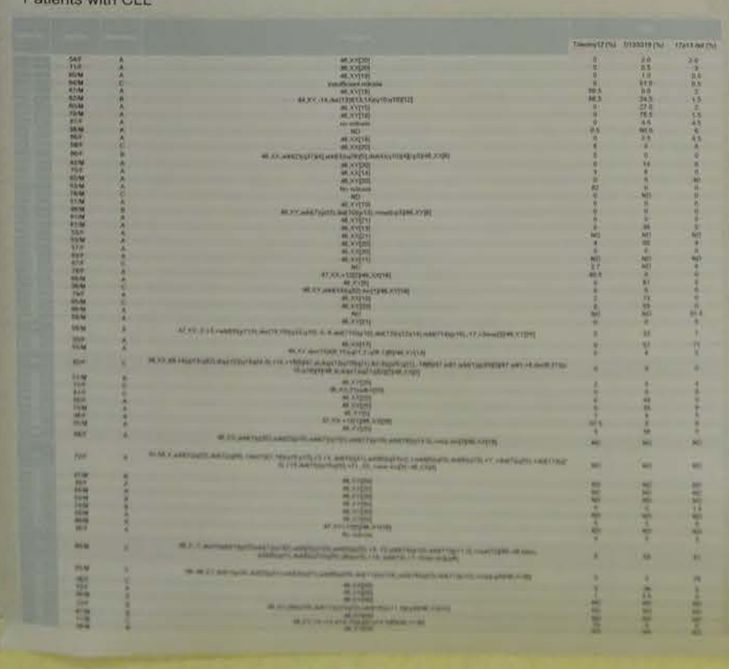
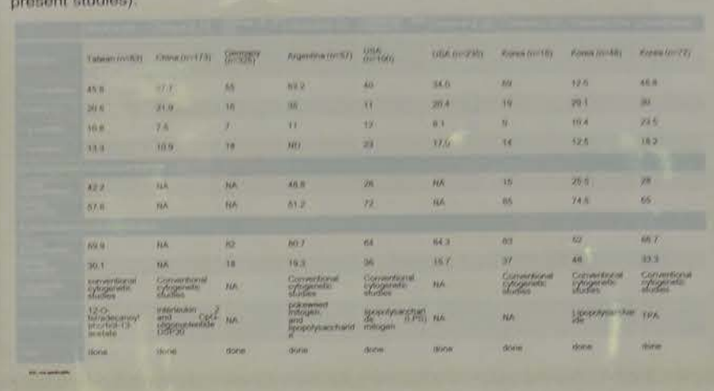


Table 2.Incidence (%) of chromosomal abnormalities in patients with CLL (previous and present studies).



Targeted sequencing

Targeted exome-sequencing analysis revealed 76 substitutions and insertion/deletions. The most common recurrent mutation (>5% frequency) was *ATM* (20.8%), followed by TP53 (14.6%), *SF3B1* (10.4%), *KLHL6* (8.3%), and *BCOR* (6.25%). The ATM_{mut}, $KLHL6_{mut}$ and $BCOR_{mut}$ were more frequent in Korean notably and the *ATM* mutation exhibited a 2-fold higher incidence (20.8% vs. 9%) in Koreans compared to Caucasians. (Fig 1, Table 3, Fig 2)

Fig 3. Genomic landscape of the Korean patients. (A) Frequency of gene mutations broken down by CLL stage. (B) Number of mutations according to the Binet stage. (C) Number of mutation sites in each gene that occur in more than 5% of the population.

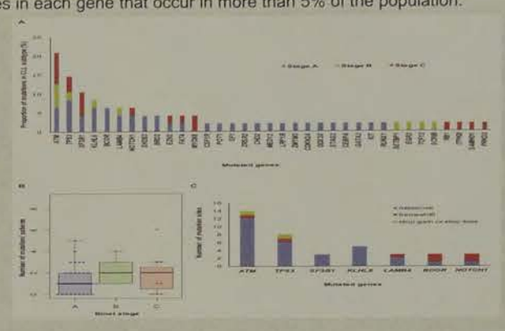


Table 3. Comparison of frequencies of mutated genes (%) between Caucasian and Korean populations

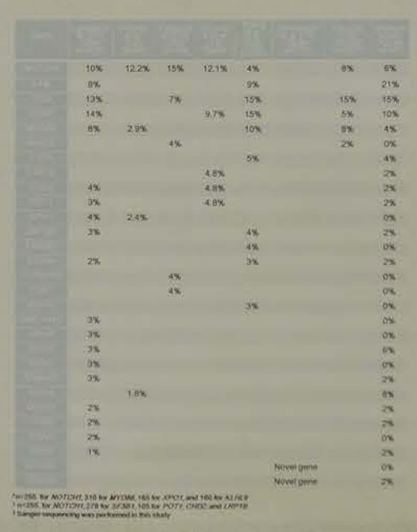
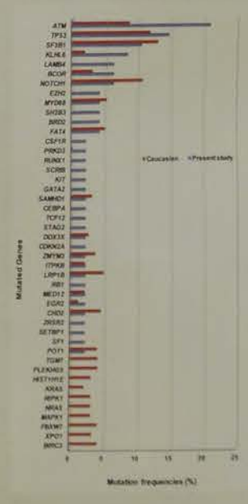


Fig 2. Comparison of mutation frequencies (%) between Caucasian and Korean population



Conclusions

The mutation profile of Korean CLL patients differed from that of Caucasian CLL patients, but their cytogenetic aberration profiles were similar. Novel mutations discovered in this study must be validated through large cohort studies and may offer clues to the mechanisms underlying the ethnic difference in CLL incidence.

References

Hematology
PC-19

COMPARISON OF MEASUREMENTS IN THE TWO POSITIONS AND SUITABILITY OF ERROR MESSAGE OF THE PFA-100

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Background

The platelet function analyzer (PFA)-100 is an in vitro system for measuring platelet function in citrated whole blood. We have wondered whether retesting by error messages is suitable and experienced a difference between measurements in the two positions of the instrument since one follows the other. The purpose of this study was to investigate accordance rate of repeated test results from abnormal values following simultaneous installation and to solve a difference in the test results of both positions.

Method 1

A total of 10,956 patient samples -5,696 male and 5,260 female- were obtained and checked the suitability of Reportable and Unreportable message.
Unacceptable specimens, Hematocrit<35%, Platelet <150*10^9/L were included.

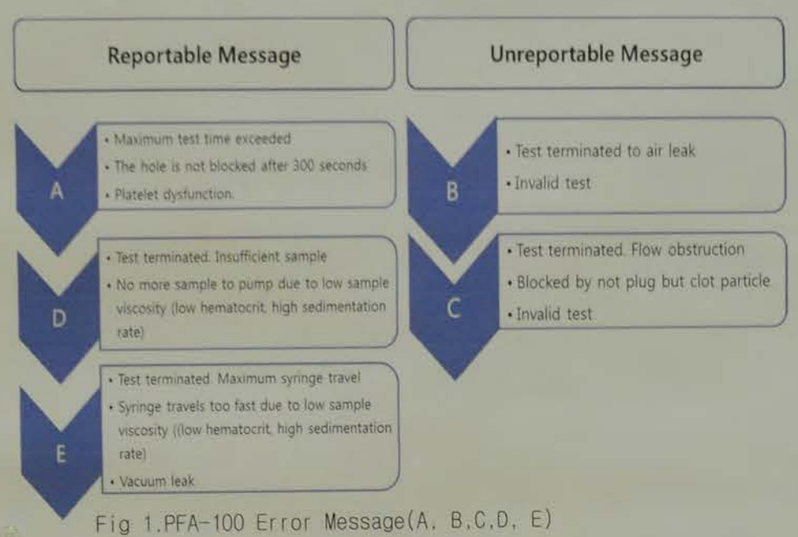

Fig 1.PFA-100 Error Message(A, B,C,D, E)

Method 2

If measuring result were prolonged in both A & B positions, the sample in B position is reexamined. In case the patient was not anemic, thrombocytopenic or had no prolonged histories, we retested with single test in the A position for the prolonged samples in B position, and compared the two results.

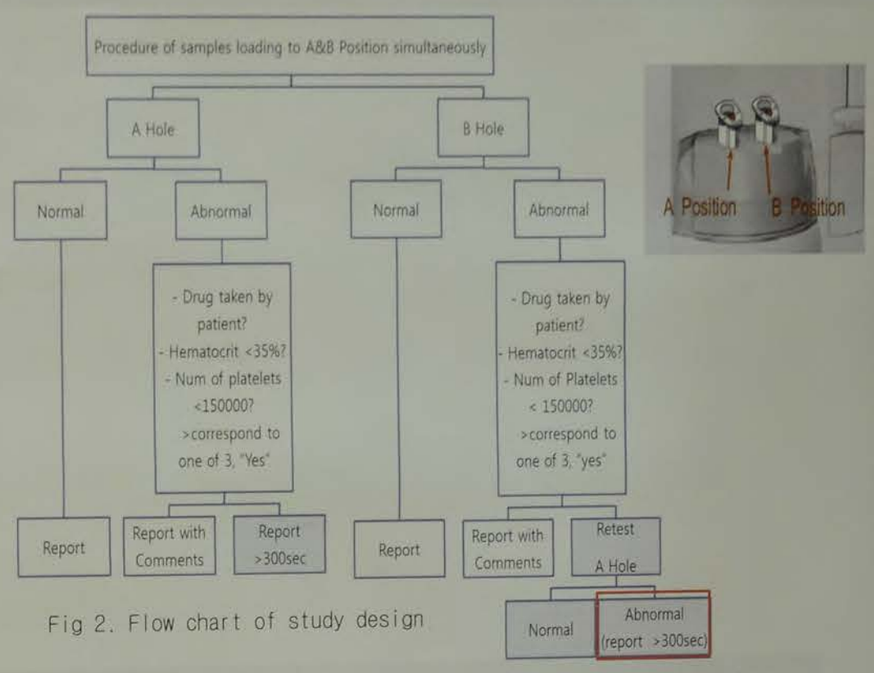

Fig 2. Flow chart of study design

Statistics

Applied SPSS 12.0 and Chi-square including frequency rate. In case Chi-square's expected frequency is too small, two-tailed test was conducted to check inaccurate result of the test by using Fisher's exact test and the significance level was set to be $p<0.05$

Result

1,544 samples (14.1%) showed prolonged CTs. 97.8% among those with error messages appeared any reportable message, showing high relevance between initial testing and retesting. 17.2% of B position samples with CT prolongation in both positions was changed to within the reference range.

Table 1. Patients characteristics of CEPI

Characteristics		Value	Total
Age	≤ 40 year	4719 (43.07%)	10956
	> 40 year	6237 (56.93%)	
Gender	Male	5696 (52%)	
	Female	5260 (48%)	
Normal	60-180 sec	9412 (85.9%)	
Prolonged		1544 (14.1%)	
	A	594 (5.4%)	
	C	13 (0.1%)	
	D	81 (0.7%)	
	E	143 (1.3%)	
	183-299 sec	713 (6.6%)	

(Hct <35% & Platelet count 150*10^9/L included)

Table 2. Comparison of error messages between initial and recheck test

Initial	Rechecked						P*
error	Reportable message			Unreportable message		전체	
message	A	D	E	B	C		
A	145 (78%)	1 (0.5%)	3 (1.6%)	-	0 (0.0%)	149 (80.1%)	<0.001
D	10 (1.6%)	6 (3.2%)	3 (1.6%)	-	0 (0.0%)	19 (10.2%)	
E	5 (2.7%)	0 (0.0%)	9 (4.8%)	-	0 (0.0%)	14 (7.5%)	
B	-	-	-	-	-	-	
C	3 (1.6%)	0 (0.0%)	0 (0.0%)	-	0 (0.0%)	4(2.2%)	
전체	163 (87.6%)	7 (3.8%)	15 (8.1%)	-	0 (0.0%)	186 (100%)	

* Chi-sqare test

Table 3. B-position results for the sample that have been deemed abnormal after simultaneously check on both A & B positions

		Post-stage				P*
		-182 sec	183-299 sec	≥300	전체	
Pre-	183-299 sec	1 (1.1%)	7 (7.5%)	5 (5.4%)	13 (14.0%)	0.001
stage	≥300	15 (16.1%)	7 (7.5%)	58 (62.4%)	80 (86%)	
전체		16 (17.2%)	14 (15.1%)	63 (67.7%)	93 (100%)	

* Chi-sqare test
+ Pre : Samples of abnormal result at A & B position following simultaneous installation
Post : Only B position re-examination (normal expected samples that have no history problem)

Conclusion

Reportable message(A,D,E) sample was 182 case among total 186 cases and it led to the conclusion that there is no need for retest which requires a lot of reagent costs. If message C appeared in case of flow obstruction, platelet aggregation was confirmed under microscopy and so it is recommended to be reexamined with new sample promptly. Message B which appears in case of air leak had not been shown because vacuum was checked by 'self test' every morning.
B Position' s reexamination is necessary in case both A position & B position shows abnormal results after loaded at the same time, and this is because A position test is in progress for more than 300 seconds, It was assumed that the sample in B position is coagulated while standing by and it led to inaccurate test result.

Frontier Spirit

Hematology

PC-21

Refining Reporting Results of Body Fluid Analysis for Clinical Purpose

Commenting Summarization of Significant Results for Reporting Results to Highlight Clinical Relevant

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Introduction

The intended purpose of body fluid cellular analysis is for the characterization of inflammatory, infectious, malignant neoplastic, and immune alterations. For those purposes, separate aliquots of body fluids are frequently analyzed simultaneously in the hematology and core laboratory, the microbiology or the cytology laboratory. Lacking the policy to correlate these results in many of laboratories limits the clinical usefulness of body fluid analysis, however body fluids cellular analysis performed by hematology laboratories still considered as a most efficiency screening test to discriminate a transudate or an exudate for its inexpensive cost and speedy turnaround time. The hematology laboratory offers body fluid results which mainly include protein (Pandy's test and Rivalta test), cellular count, and leukocyte differential count. According to CLSI H56-P, it is important to understand that body fluid differential counts are not an appropriate screening for malignant diagnosis. Let alone for malignant neoplasm screening, the reference intervals of leukocyte or deferential count are not only variant but also complicate. For decades, the equivocal reference intervals of each cellular element count and incomparable semi-quantitative protein results indeed acquire much more difficulty for clinicians to diagnosis. This study more focuses on infectious relevant evaluation, for this purpose we are trying to reevaluate the appropriateness of the reference intervals of leukocyte count of each serous fluid, and to discuss the clinical effectiveness of Rivalta test or leukocyte differential count. Which is more likely chosen as the targeted parameter? The other purpose of this study is trying to acknowledge our reporting personnel to summarize this significant clinical relevant information on comment field as necessity as reporting an accurate result.

Materials & Methods

Hemocytometer (Neubauer counting chamber), hemocytometer coverslip, 3% acetic acid, acidified crystal violet stain were used for cellular counting. Slides for microscope examination were prepared by Cytocentrifugation and Wright-Giemsa stain (Hematek). While differential count performed manually, for those other nucleated cells were found and the number was over 5 in 100 leukocytes, the leukocyte count will be corrected.

The 622 cases for body fluid were collected in March 2016, and all results released by the hematology laboratory were included in this study. We try to tract microorganism isolation results of those aliquots simultaneously analyzed for bacterial cultures. Three parameters which included results of protein(semi-quantitative), leukocyte, and differential count were used to evaluate the association between each of them with culture positivity. The most relevant one will be chosen as the targeted parameter.

Moreover in order to confirm effectiveness of accurate diagnosis of the targeted parameter, we also analyze 6,837 cases collected from Jan. 2015 to Jun. 2016, whose specimens are analyzed simultaneously in the hematology laboratory and the microbiology laboratory.

Summarized statistics and Student's unpaired t-test were performed using the SAS Enterprise Guide software (version #6.1) and GraphPad Prism (version #5.0). The cutoff value percentage of neutrophil (relative neutrophil count) and ROC curve were performed using SigmaPlot (version #12.0). Using SigmaPlot software calculates sensitivity and specificity by defining the results of body fluid culture as gold standard, and other parameter like protein test, leukocyte count and percentage of neutrophil as variables.

Results

The total 622 specimens include 239 pleural fluids, 203 ascites, 134 CSFs, 43 synovial fluids and 13 pericardial fluids, in which 5 specimens could not be performed cellular analyses due to serious cellular lysis. The cases of leukocyte count surpass reference intervals (5 cells/μL for CSF and 1,000 cells/μL for others) comprised 55 pleural fluids, 35 ascites, 13 CSFs, and 28 synovial fluids. Suspected malignant neoplastic cells (atypical cells) were found in 29 cases, of which include 20 Pleural fluids, 7 ascites and 2 CSFs. Other findings in microscopic examinations were 13 cases of microorganisms and 4 synovial fluids of monosodium urate crystals.

In total 622 specimens, 34 cases were simultaneously requested for bacteria cultures and were proved positive culture results, but due to inappropriate maintained only 28 cases could acquire complete cellular results as mentioned. The results from hematology laboratory and microbiology laboratory were integrated as Table 1.

Table 1

Specimen \ Test	Frequencies of results over reference intervals for each test					Microorganism culture (+) *
	Semi-quantitative protein (+)	Leukocyte count high (>5 cells/μL for CSF ; >1000 cells/μL for others)	Microscope			
			Neutrophilia (Neu>60%)	Microorganism present	MSU present	
Pleural fluid (Total:239 cases)	113	55	43	3	-	9
Ascites (Total:203 cases)	17	35	72	10	-	18
CSF (Total:134 cases)	27	13	7	-	-	1
Synovial fluid (Total:43 cases)	-	28	26	-	4	-
Pericardial fluid (Total:3 cases)	-	-	-	-	-	-

* For those aliquots simultaneously analyzed in the microbiology rather than all cases.

We also found 27 cases showed high percentage of neutrophil (56%~99%) in total 28 culture positive cases, no matter the isolations were bacterial or yeast . It may propose that relative neutrophil count seems more likely associated with bacterial infection.

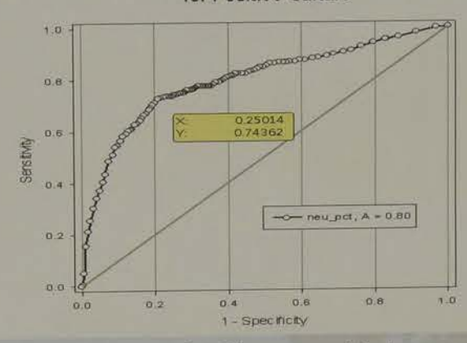
In order to evaluate if cases of protein positivity posses the same characterization of neutrophil percentage associated with bacterial infection. The results of 6,837 cases from Jan. 2015 to Jun. 2016 provided by the hematology and microbiology laboratory were collected for further analysis. Chi-square test was used to evaluate the association between protein test and bacterial culture. Table 2 showed the semi-quantitative protein tests may not be proposed relevant to bacterial culture positivity (p=0.159).

Table 2

N=6,837		Culture		Total
		Positive	Negative	
Protein test Positive	Count	199	3,504	3,703
	Expected Count	186	3,517	3,703
	% of Total	2.9%	51.3%	54.2%
Protein test Negative	Count	145	2,989	3,134
	Expected Count	158	2,976	3,134
	% of Total	2.1%	43.7%	45.8%
Total	Count	344	6,493	6,837
	Expected Count	344	6,493	6,837
	% of Total	5.0%	95.0%	100.0%
Chi-square test p-value=0.159				

In contrary, Fig. 1 showed the percentage of neutrophil seemed to be more effective parameter for bacterial infection diagnosis. Besides, it indicated the point of 60% neutrophil with 74% sensitivity and 75% specificity seems to be the most appropriate cutoff value for accurate diagnoses.

Fig.1 ROC Curve of Predicted Probability % Neutrophil for Positive Culture



Based on this cutoff value of 60% neutrophil, box-and-whisker plots (boxplots) are tried to redefine the reference intervals of leukocyte count for ascites and pleural fluid current used in our laboratory. Fig. 2 shows that ascites leukocyte count of 500 cells/μL set for cutoff value is proposed to be more sensitive for screening infection compared with leukocyte count of 1,000 cells/μL current used. As to pleural fluids, there is no significant range of leukocyte count could be considered as cutoff value demarcated by 60% neutrophil count (Fig.3).

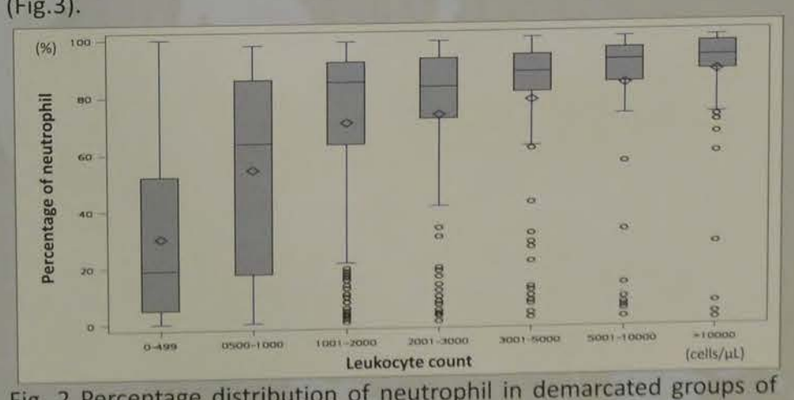


Fig. 2 Percentage distribution of neutrophil in demarcated groups of leukocyte count for ascites.

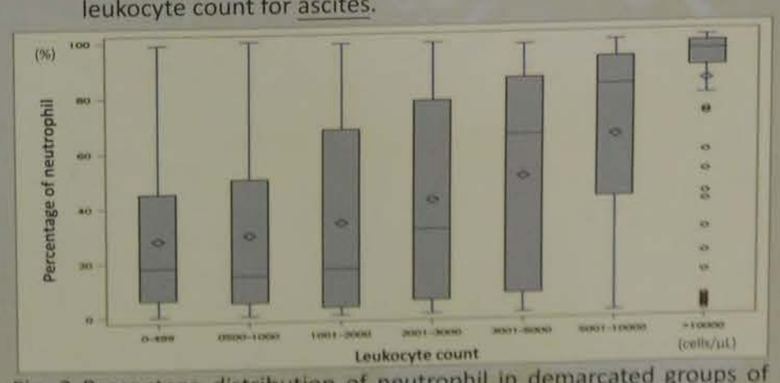


Fig. 3 Percentage distribution of neutrophil in demarcated groups of leukocyte count for pleural fluid.

Discussion & Comment

Neutrophil predominant (56%~99%) were found in 27 cases of total 28 culture positive cases, no matter bacterial or yeast were isolated, as were in 4 cases with MSU findings. The only case with positive isolation of Escherichia coli showed lower relative neutrophil count (21%), which came from an end-stage malignant neoplasm patient. In this study, 60% neutrophil count possesses 74% sensitivity is revealed that 60% neutrophil may potentially be proposed as a potent parameter for bacterial infection diagnosis, if data of more bacteria infection cases are accumulated and more uncovered variables are incorporated in the future.

After study, the leukocyte count intervals for ascites has been changed from 1,000 cells/μL to 500 cells/μL, and reporting staff are mandated to indicate neutrophil predominant in the comment field of the report form if the relative neutrophil count is over 60%.

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Hematology

PC-22

Establishment of novel flow cytometric test for Adult T-cell leukemia (ATL) and its clinical application

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【Background】

Human T-cell leukemia virus type 1 (HTLV-1) is present throughout the world, and endemic especially in the Southwestern Japan, sub-Saharan Africa, South America, the Caribbean area, Middle East and Melanesia. The total number of HTLV-1 carriers was estimated to be 10-20 millions. Clinically, ATL is classified into four subtypes: smoldering, chronic, lymphoma, and acute type. The former two are indolent while the latter are aggressive. Although the incidence rate of ATL in HTLV-1 carriers is not so high, the prognosis of acute- or lymphoma-type ATL is extremely poor. HTLV-1 carriers and indolent ATL patients are followed up carefully without therapies, and evaluation of disease status is very important since progression to aggressive ATL can't be expected in advance.

There were no clinical examinations which could evaluate disease status easily and exactly. One of the standard assays commonly used for the evaluation is morphological counting of abnormal lymphocytes, but discrimination of HTLV-1 infected cells from other lymphocytes, especially from reactive lymphocytes, is difficult. Due to the difficulty and requirement of experience, inter-examiners differences are known to be large. Another standard assay is HTLV-1 proviral load (PVL) quantified by PCR. The problem is that 5 to 7-fold differences in the results between laboratories were reported. Moreover, as the method takes time and effort, applying this in general clinical laboratory is difficult.

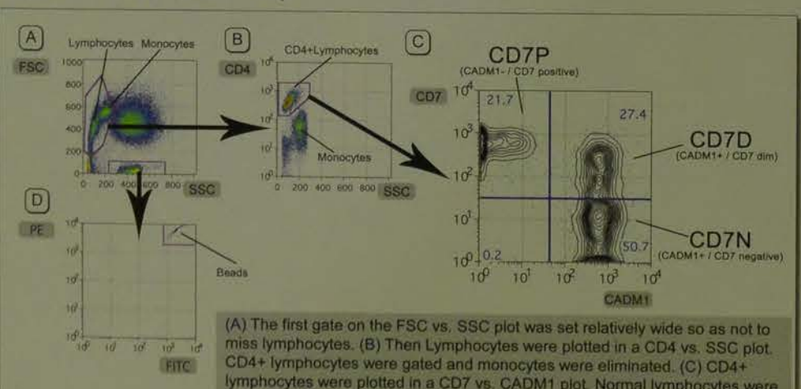
We have assessed a number of samples from HTLV1-infected patients using 12-color flow cytometry, and tried to establish a simple clinical laboratory test which is useful for clinical practice.

【Materials】

Peripheral blood samples were collected from HTLV-1 carriers and ATL patients who attended or were admitted to the Research Hospital at the Institute of Medical Science, The University of Tokyo (IMSUT) between January 2013 and March 2016. Some of patients were transferred to our hospital after a few courses of chemotherapy. This study was approved by the Research Ethics Committee of IMSUT, and written informed consent was obtained from all patients in accordance with the Declaration of Helsinki. All patients were diagnosed according to Shimoyama's criteria. 163 samples from 58 patients were collected before treatment or just before a course of chemotherapy.

【Methods / Clinical Laboratory Testing】

Peripheral blood was obtained in Vacutainer Hemogard Plus tubes (BD Biosciences, San Jose, CA, USA) by conventional venipuncture. The volume necessary for our method was only 200 µl, and the rest of peripheral blood samples used for routine laboratory tests were applied for measurement. For quantification, a ProCOUNT method using Trucount tubes (BD Biosciences), in which a known number of fluorescent reference beads are included, was adopted to measure the absolute number of CD4+cells. In advance, anti-CADM1 antibodies were labelled with phycoerythrin (PE). Fluorescently labeled antibodies, consisting of fluorescein isothiocyanate (FITC)-CD3, allophycocyanin (APC)-CD7, PerCP-CD4, and PE-CADM1, were mixed in a tube. Then, 100 µl of whole peripheral blood were added to the tube and mixed well. The cells were stained for 15 min at room temperature. After staining, 1 ml of Cell Lysis Buffer was added to lyse red blood cells. After 15 min, the sample was once washed and centrifuged, After vortexing gently for 10 s, we analyzed samples with a FACSCalibur flow cytometer (BD Immunocytometry Systems, San Jose, CA, USA) as soon as possible. Flow cytometry data were analyzed with FlowJo 9.9 (Treestar, San Carlos, CA, USA).



(A) The first gate on the FSC vs. SSC plot was set relatively wide so as not to miss lymphocytes. (B) Then Lymphocytes were plotted in a CD4 vs. SSC plot. CD4+ lymphocytes were gated and monocytes were eliminated. (C) CD4+ lymphocytes were plotted in a CD7 vs. CADM1 plot. Normal lymphocytes were mainly enriched in CADM1-/CD7+(CD7P) fraction, and HTLV-1 infected cells were enriched in CADM1-/CD7dim(CD7D) and CADM1+/CD7Negative(CD7N) fraction. (D) Reference beads were gated in the PE vs. FITC plot, according to the manufacturer's instructions.

<Fig.1> Classification of CD4+ lymphocytes into CD7P/D/N and gating of beads for quantification.

【Results① Basic】

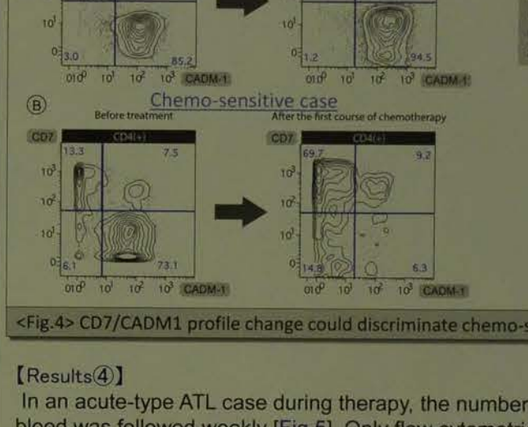
CD4-positive lymphocytes can be mainly classified into CD7P, CD7D, and CD7N fractions. HTLV-1 infection and clonality of each fraction was evaluated [Fig.2].

CD4+ cells in patients infected with HTLV-1 can be classified mainly into three groups: CADM1^{negative}/CD7^{positive} (CD7P), CADM1^{positive}/CD7^{dim} (CD7D), and CADM1^{positive}/CD7^{negative} (CD7N). Assessment of clonality in the context of the T-cell receptor Vβ repertoire and HTLV-1 proviral load(PVL) showed that HTLV-1 infected ATL cells are highly concentrated in CD7D and CD7N fraction.

<Fig.2> CD7D(D) and CD7N(N) cells are HTLV-1 infected cells.

【Results② Correlation】

The number of HTLV-1-infected cells measured by this flow cytometric test was compared with the results of conventional standard tests. The number of CD4+CADM1+ (CD7D and CD7N) cells measured by cytometry had a strong linear correlation with the number of abnormal lymphocytes measured microscopically, or with the product of the white blood cell count and HTLV-1 proviral load [Fig.3].



(A) The number of CD4+CADM1+ (CD7D+CD7N) cells measured by flow cytometry had a strong linear correlation with the product of the white blood cell count and HTLV-1 proviral load.
(B) The number of CD4+CADM1+ cells measured by flow cytometry had a strong linear correlation with the number of abnormal lymphocytes measured microscopically. The linear regression coefficient was very close to 1.00.
(C) Soluble IL-2R had a significant relation with the number of HTLV-1-infected cells, but the correlation was low (Spearman ρ =0.392).
(D) LDH didn't have a significant relation with HTLV-1-infected cells (Spearman ρ =0.034).

<Fig.3> Comparison of the results between flow cytometric and conventional standard assays.

【Results③】

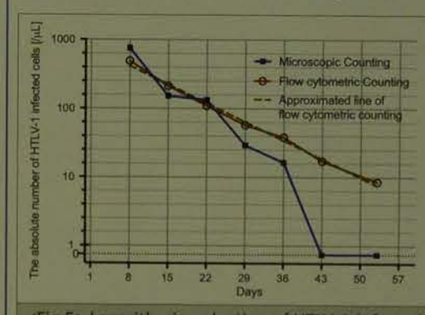
After following patients, we found that the frequency of CD7D and CD7N cells among CD4+ cells changed during chemotherapy, which reflected differences between chemo-sensitive and chemo-resistant cases [Fig.4].

(A) A patient with an almost unchanged CD7/CADM1 profile after the first course of chemotherapy had an unfavorable prognosis.
(B) In contrast, a patient showed a good clinical response to chemotherapy exhibited marked changes in the CD7/CADM1 profile after only one course of chemotherapy.

<Fig.4> CD7/CADM1 profile change could discriminate chemo-sensitive and resistant cases.

【Results④】

In an acute-type ATL case during therapy, the number of ATL cells in the peripheral blood was followed weekly [Fig.5]. Only flow cytometric test detected MRD (minimum residual disease) and showed logarithmic reduction of HTLV-1-infected ATL cells.



As leukemia cells can be reduced logarithmically, logarithmic graph of flow cytometric counting showed an almost straight line, while microscopic counting sways and could not detect ATL cells after 43days.

<Fig.5> Logarithmic reduction of HTLV-1-infected ATL cells

【Summary】

We have established a new clinical test which can accurately quantify HTLV1-infected ATL cells using simple four-color flow cytometry, and used the test in clinical practice.

【Merits in this clinical laboratory testing】

✓ *Patient Friendly*

As this test requires only 200 µl of residual peripheral blood samples, no additional blood drawing is necessary.

✓ *Technician Friendly*

The method is easy and not time-consuming.

✓ *Accurate and Speedy*

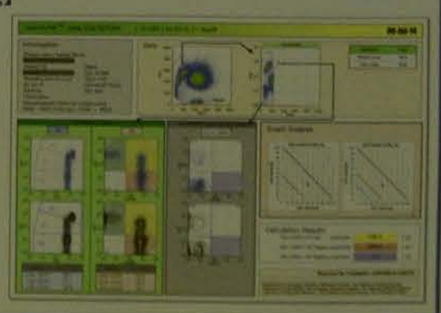
Cytometer can evaluate more than 10 thousands of cells accurately and promptly.

✓ *Sensitive (MRD detection)*

Cytometry is sensitive enough to detect MRD (minimal residual disease).

✓ *Prognostification*

This test can distinguish chemo-sensitive cases from resistant cases.



【Conflict of Interest】 We have no potential COI to disclose.

Hematology

PC-23

DIT 동의과학대학교 DONG-EUI INSTITUTE OF TECHNOLOGY

Effect of pH levels on Blood Cell Morphology *in vitro*

- The important maintenance of pH levels to play a key roles in Blood Cells -

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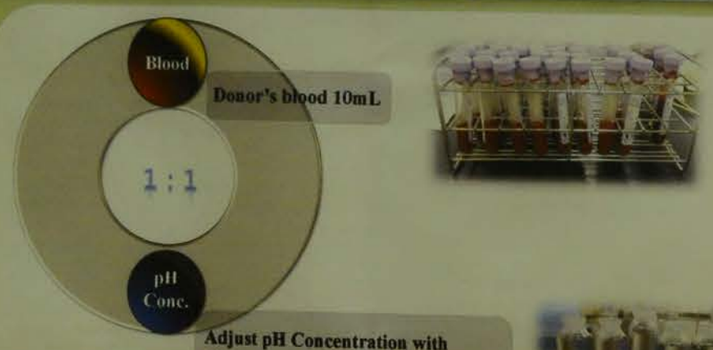
Abstract

Blood pH level is very important in the human body to maintain homeostasis. This study shows the effect of pH level on Blood Cell Morphology in vitro. Blood sample were collected from healthy college students in early 20's. We studied blood cell morphology (esp. WBC and RBC) in each pH levels from acid (pH 5.0) to alkali (pH 9.0) conditions and adjusted to pH using 0.1N NaOH and 0.1N HCl in diluents. At first each samples were analyzed by Sysmex XS-1000i (Japan) and stained Wright's stain for microscopy. As a results at pH 8.0 RBC showed rouleau formation and at pH 9.0 RBC showed rouleau formation strongly, some WBC showed naked nucleus. While at pH 6.0 RBC changed from normal shape to tear drop and burr cell types and WBC showed naked nucleus at the same pH 9.0. At pH 5.0 lots of RBCs showed schistocytes and spur cells. In conclusion Blood cells are sensitive in pH variation and especially RBC was more sensitive than WBC. This study shows how important pH conditions in our body and blood cells to play s a key roles in their functions.

Materials & Methods



Results

1. RBC morphology

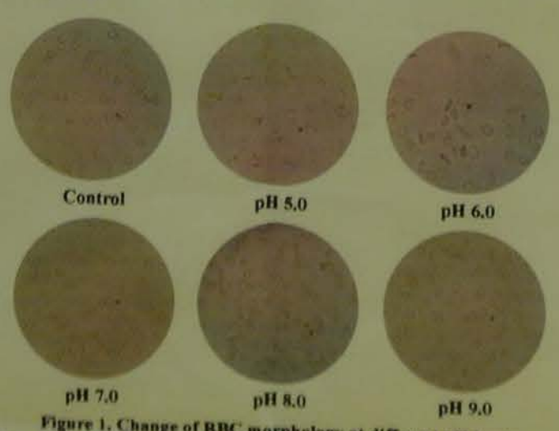
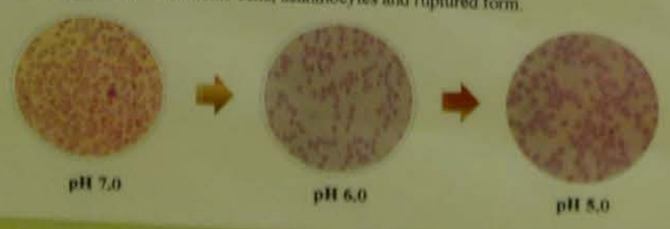


Figure 1. Change of RBC morphology at different pH levels.
This shows the effect of pH level. RBC's changed their shape at acid and alkali levels compared with control and pH 7.0.

1) Acid condition
RBC's changed and showed Burr cells, acanthocytes and ruptured form.



2) Acid condition
RBCs changed smaller than normal condition and formed rouleaux, and ruptured.

2. WBC morphology

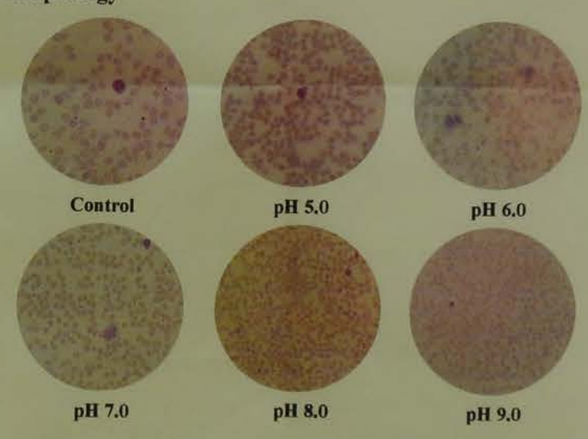
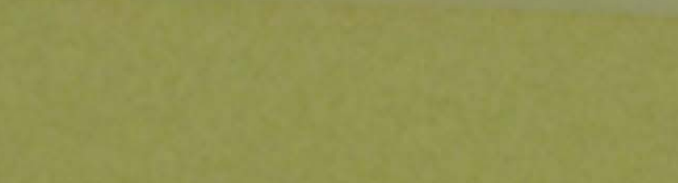


Figure 2. Change of WBC morphology at different pH levels.
This shows the effect of pH level. WBCs changed their shape at acid and alkali levels compared with control and pH 7.0.

1) Acid condition
WBCs cell membrane changed and ruptured like ghost cell.



2) Alkali condition
WBCs nuclear shapes changed.

Conclusion

This study show that blood pH is very important to maintain the blood cells shape and characteristics. RBC shape changed from biconcave(nuts shape) to burr cell, tear drop, ovalocyte, and ruptured as pH levels lowed. This means that low pH levels can influence the RBC's functions, especially gas exchange due to break down cell membrane. While WBC has less influenced rather than RBC. But at lower pH level WBC showed change nuclear shapes and cell membrane was broke.

Generally blood maintain about pH 7.4. But CBC analysis equipments have a few reagents to analyzes blood cells which is cell counts, shapes and differentiation. So technologist must keep the expiration date of reagents especially diluent solutions and protect contaminations in the laboratory.

Hematology
PC-24

...ld Congress of Biomedical Laboratory Science (IFBLS). August 31–September 4, 2016, Kobe, Japan.

Usefulness utility of leukocyte and platelet parameters for rapidly discriminating bacteremia and urinary tract infections.

長庚 高雄

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Background

- **Urinary tract infection (UTI)** is one of the leading bacterial infections among adults and it can occur in any part of the urinary tract. UTIs are a common infection that affect more women than men. The most common symptoms are burning with urination, frequent urination, fever and flank pain. An untreated UTI may spread to the kidney, causing more pain and illness. It can also cause sepsis. Most often, UTIs are treated quickly and effectively with antibiotics, the infection becoming a distant memory. Unfortunately, not all UTIs are treated quickly and some aren't even identified quickly, particularly in people who have limited or no sensation below the waist or who are unable to speak for themselves. Particularly troublesome is the increasing resistance to broad-spectrum antibiotics, in particular to fluoroquinolones and cephalosporins, due to an overconsumption of these two groups and the parallel development of co-resistance to other antibiotics (collateral damage). It is essential to consider the local microbial environment and resistance pattern as well as risk factors for harbouring resistant microbes in individual patients..
- **Bacteremia** is the presence of viable bacteria in the blood stream. Bacteremia elicits a vigorous immune system response. The constellation of findings related to this response (such as fever, chills) is referred to as urinary tract infection. Bacteremia may cause metastatic infections, including pneumonia, brain abscesses, cellulitis, endocarditis. Transient bacteremia is often asymptomatic but may cause fever. Development of other symptoms usually suggests more serious infection, such as sepsis or septic shock, possibly resulting in death. Early treatment of bacteremia with an appropriate antimicrobial regimen appears to improve survival.
- Despite improvements in urinary tract infection treatment modality and development of various biomarkers used for early detection, urinary tract infection and bacteremia remain important cause of mortality, especially in intensive care unit patients. Common signs and symptoms of UTIs and bacteremia include fever, thrombocytopenia, and neutrophilia. However, microbiological cultures; the conventional gold standard diagnostic method, are often time consuming. Current biomarkers, such as lactate, procalcitonin, TNF-α, IL-6, IL-1β and C-reactive protein (CRP), used for the detection of infection can reflect the pathologic conditions associated with bacteremia development and are useful in sepsis diagnosis or assessment of sepsis severity; however, performance of these biomarkers varies. It is important that a rapid and sensitivity biomarker be discriminate patients with bacteremia from UTIs patients.
- Reliable parameters are analyzed on WBC and platelet indices that easily obtain by cell counting devices, facilitate the diagnosis process. At present, cell counters are widely used in routine practice, so screening can be done without additional costs to medical systems.
- Inflammation induces coagulation abnormalities resulted from platelet activation and leukocytosis or neutrophilia can cause end-organ damage in sepsis pathophysiology. It is possible that the parameter reflecting platelet production and activation, specifically the immature platelet fraction that indicates the percentage of reticulated platelets.

Purpose

We evaluated the performance of hematological indices by cell counters, such as immature granulocyte fraction (IG%), immature reticulocyte fraction (IRF), immature platelet fraction (IPF%), mean platelet volume (MPV), platelet distribution width (PDW) and high-fluorescent lymphocyte counts (HFLC%) as valid bacteremia biomarkers focusing on three points: (1) the discrimination of bacterial infection patients from non-bacterial infection patients with fever; (2) the discrimination of bacteremia patients from UTIs patients; and (3) the assessment of bacteremia or sepsis severity in comparison with non-hematological biomarkers such as lactate, procalcitonin and C-reactive protein (CRP).

Methods

1. Our study included 3 groups, namely, 357 subjects with UTIs, 318 subjects with bacteremia, and apparently 298 hospital employees.
2. We used the Sysmex XE-5000 automated hematology analyzer and analyzed with full hemocytometric parameter profile. For each sample, PCT, CRP, and lactate were measured.
3. The Pearson correlation and ANOVA were calculated; ROC curve analysis was used to determine their diagnostic performance.

Results

1. Bacteremia and UTIs patients showed significantly higher IPF% ($p < 0.001$), HPLC% ($p < 0.001$), IG% ($p < 0.001$), IRF% ($p = 0.010$), WBC counts ($p = 0.021$), neutrophils% ($p < 0.001$), lactate ($p = 0.032$), procalcitonin ($p < 0.001$) and C-reactive protein ($p < 0.001$) than non-infectious patients. Additionally, bacteremia patients showed significantly higher IPF% ($p < 0.001$), HPLC% ($p < 0.001$), IG% ($p < 0.001$), IRF% ($p = 0.030$), WBC counts ($p = 0.031$), neutrophils% ($p < 0.001$), C-reactive protein ($p < 0.001$), lactate ($p = 0.042$) and procalcitonin ($p < 0.001$) than UTIs patients.

	bacteremia (n=318)	UTIs (n=357)	Non-infectious (n=298)	P value
PCT (ng/mL)	21.8 ± 12.8	17.1 ± 9.8	0.3 ± 0.1	<0.001
CRP (mg/L)	81.8 ± 21.7	79.8 ± 22.9	12.7 ± 9.8	<0.001
lactate (mmol/L)	3.1 ± 0.5	1.4 ± 0.6	0.5 ± 0.7	0.042
WBC (*1000/μL)	18.8 ± 4.7	13.2 ± 6.3	8.7 ± 2.9	0.031
Neu (%)	89.1 ± 10.9	72.8 ± 9.7	61.9 ± 5.8	0.043
IG%	0.9 ± 0.3	0.5 ± 0.1	0.1 ± 0.1	<0.001
HFLC (%)	0.9 ± 0.3	0.4 ± 0.3	0.1 ± 0.1	<0.001
PLT (*1000/μL)	197 ± 21.3	230 ± 23.8	256 ± 27.5	0.043
MPV (fL)	10.9 ± 0.3	10.3 ± 0.2	13.4 ± 0.3	<0.001
plateletcrit (%)	0.21 ± 0.03	0.24 ± 0.06	0.31 ± 0.09	<0.001
IPF(%)	7.1 ± 0.8	1.4 ± 0.7	1.7 ± 0.9	<0.001
PDW (fL)	12.1 ± 0.5	10.9 ± 0.6	13.8 ± 0.7	0.031
FRC (%)	0.26 ± 0.11	0.13 ± 0.04	0.08 ± 0.02	0.013
IRF (%)	10.8 ± 0.8	8.6 ± 0.7	5.7 ± 0.9	0.030

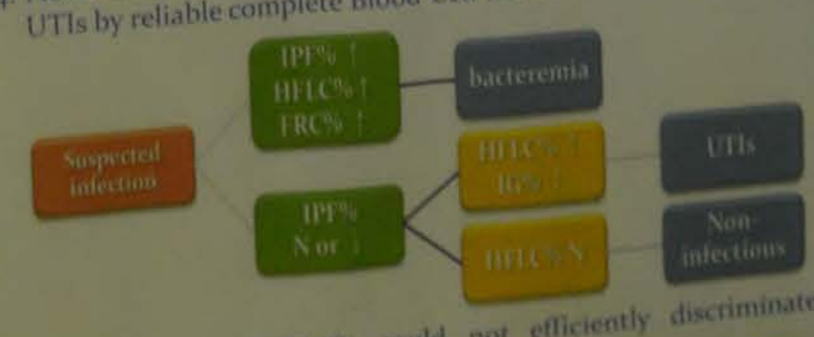
2. Bacteremia patients with complicated sepsis or septic shock showed significantly higher lactate ($p<0.001$) and IPF% levels ($p=0.032$), lower platelet counts ($p<0.001$) and MPV ($p<0.001$) than those with uncomplicated sepsis. However, patient age, PCT, CRP, IG, IRF, WBC counts, hemoglobin and WBC differential counts were not significantly different between the two patient subgroups. The type of bacteremia was not significantly different between the two patient subgroups.

	GPC+GPB	GNB	P value
HFLC (%)	0.7 ± 0.4	0.5 ± 0.4	0.534
MPV (fL)	10.5 ± 0.2	10.3 ± 0.3	0.571
plateletcrit (%)	0.23 ± 0.06	0.24 ± 0.07	0.731
IPF(%)	6.7 ± 0.9	6.4 ± 0.3	0.813
PDW (fL)	11.1 ± 0.6	10.9 ± 0.4	0.435
FRC (%)	0.26 ± 0.17	0.28 ± 0.19	0.541

3. Among all biomarkers, both IPF% plus HPLC% performed best, with both their specificities and PPVs being very high. However, their accuracies were not as high as their specificities and PPVs. The IPF showed comparable performance to PCT, IG%, LA, and IG, and showed significantly better performance than the CRP ($p=0.007$). The IPF% plus HPLC% and IG% demonstrated the highest sensitivity (98.1%, 95% confidence interval 93.3-99.2%) and accuracy (95.7%, 95% CI 93.1-99.0%) among sum of three biomarkers when 5% was applied as a cutoff value.

Biomarker	Sensitivity	Specificity	AUC	95% CI
PCT	91.7%	98.3%	0.805	0.819-0.935
CRP	76.8%	64.8%	0.712	[illegible]
IPF%	86.8%	92.1%	0.837	0.791-0.884
HFLC%	91.8%	93.1%	0.849	0.817-0.947
IG%	80.7%	88.7%	0.811	0.760-0.837
IPF%+HFLC%+IG%	98.1%	95.7%	0.978	0.933-0.997

4. Flowchart of rapid discrimination between bacteremia and UTIs by reliable complete Blood Cell Indicates.



5. The IPF% and HFLC% could not efficiently discriminate bacteremia severity (AUC=0.599).

Conclusions

1. The IPF% and HFLC% possessed high sensitivity and accuracy. We demonstrated that the IPF% and HFLC% performs better than the LA, PCT, and CRP in the discrimination of bacteremia patients from UTIs patients with local infection.
2. The IPF% and HFLC% did not efficiently discriminate sepsis or bacteremia severity.
3. We conclude that the advanced algorithms are rapid useful as laboratory devices for bacteremia screening.

Hematology

PC-25

Guides for manual estimation of blood slide review

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Introduction

The criteria for action following automated CBC and WBC differential analysis has been validated by hematology smear review evidence via consensus agreement for a decade. It did help maintain uniformity of automation applications among hematology laboratories. However, there was no detailed guideline developed by laboratories for reviewers. Laboratories are incline to mandate reviewers to report or even revise instrument results based on their own experience without evaluating necessity and accuracy of revision. It makes the slide reviewer incapable to ensure the consistency of review reporting, neither guarantee for more accurate. In order to enhance the consistency of manual reporting among different reviewers, this study is aimed to set the algorithm for reviewers to decide if the original results need to be revised after slide review.

Materials & Methods

1. Automated CBC and white blood cell differential analysis were performed with Beckman coulter LH780 and LH750. On the basis of our criteria, 6,938 cases of the arrested results were collected on day shifts in a period of two weeks for further evaluation.
2. Revision algorithm (table 1) was offered to our staff to comply with.
3. There were two indicator for evaluation of practicability and efficiency : questionnaires (table 2) were created for to evaluate the satisfaction of reviewers regarding application of revision algorithm; decreased turnaround time.

Table 1 Blood Smear Revision Algorithm

High-power field (HPF)	On 40x, examine blood smear for platelet clumps, microfilaria, abnormal WBCs, etc		
Viewing Area	Choose a one-cell layer area, no overlapping/crowding of cells, in "feathered edge".		
Count WBC/PLT in 5 HPF On blood smear made by LH780	RBC=300x10⁴ ‧ Plt=50/HPF ‧ WBC=2/HPF Plt =50×0.3x10⁴=15x10⁴ (within 40% → acceptable) WBC=2×0.3x10⁴×0.8=0.48x10⁴ (within 40% → acceptable)		
Coulter FLAG	Note for review	ADVIA	comment
VARIANT LYM BLAST IMM.NE2	Van Lym. Blast Imm.gran.	✓	Atypical Cells% (Band<15% need not report)
NRBC	NRBC ?	✓	✓>5/10WBC → correct WBC
PLT CLUMPING (vortex) GIANT PLT ((vortex)	PLT clumps PLT 假↓?	✓ ✓	PLT clumps→PLT false↓→ (1)
Plt estimation R* Cellular Interference	Estimate WBC/PLT PLT false↑?	✓	Estimation and delta check consistent (3) inconsistent(2)

1 ✓ : Report no Flag result of ADVIA and deal with flag-result at reviewer's discretion.
2 Don't report estimated result, usually for comment determination only, except (3)
(1) PLT clumps may result in false low PLT count
(2) RBC fragments may result in false high PLT count, recollection suggested
(3) Estimated Plt count, recollection suggested
3 DC : Only minor adjust automated hematology analyzer result be allowed. When revised DC result should record and co-sign by another medical technologist.

Table 2 Satisfaction Survey

How much do you agree with the revision algorithm?

	Strongly agree	Agree	Neutral	Disagree	Extremely disagree
WBC Estimation results complied with expect	O	O	O	O	O
PLT Estimation results complied with expect	O	O	O	O	O
WBC Estimation Method help reduce stress at work	O	O	O	O	O
PLT Estimation Method help reduce stress at work	O	O	O	O	O
Rerun by ADVIA is helpful for Van. Lym	O	O	O	O	O
Rerun by ADVIA is helpful for Blast	O	O	O	O	O
Rerun by ADVIA is helpful for Imm. Gran.2	O	O	O	O	O
Rerun by ADVIA is helpful for NRBC	O	O	O	O	O
Rerun by ADVIA is helpful for R* flag	O	O	O	O	O
Rerun by ADVIA is helpful for MCHC≧36	O	O	O	O	O
Revision Algorithm help make decision for report	O	O	O	O	O
Overall, Revision Algorithm help reduce stress at work	O	O	O	O	O

Table 3 Incidence of cause for revised

Criteria	Interception (n)	%	manual change result	Revised manually
Plt<100000/uL	1557	22.44%	44	2.83%
Mono>15%	906	13.06%	160	17.66%
WBC<3000/uL	840	12.11%	48	5.71%
Neu>90%	800	11.53%	144	18.00%
Imm.Grans	586	8.45%	110	18.77%
No DC	369	5.32%	369	100.00%
NRBC	318	4.58%	78	24.53%
Plt clumps	303	4.37%	30	9.90%
WBC>20000/uL	295	4.25%	48	16.27%
Plt *R	175	2.52%	27	15.43%
Baso>2	162	2.33%	49	30.25%
MCHC>36 g/dL	122	1.76%	60	49.18%
Hct/Hb>3.1	102	1.47%	16	15.69%
Var. Lym	101	1.46%	10	9.90%
Eosin>15%	81	1.17%	10	12.35%
Lym>65%	80	1.15%	1	1.25%
Ly blast	56	0.81%	12	21.43%
WBC*R	43	0.62%	3	6.98%
Mo blast	42	0.61%	19	45.24%
total interception	6938	100%	1238	17.84%

Results & Discussion

It was found that the results of 1,238 cases (18% of all 6938 cases) had been revised, and the revisions of 180 cases (21% of all 1238 cases) based on rerun results after actions taken. Other revisions include 825 cases of immature cells finding or no DCs from instrument, 78 cases for corrected WBC count due to NRBC, and other 155 cases of DC revision resulted from incomparable DC between instrument count and reviewers' estimation. It was found the results that 1,238 cases (18% of all 6938 cases) had been revised, and the revisions of 180 cases (21% of all 1238 cases) based on rerun results after actions taken. Other revisions include 825 cases of immature cells finding or no DCs from instrument, 78 cases for corrected WBC count due to NRBC, and other 155 cases of DC revision resulted from incomparable DC between instrument count and reviewers' estimation (table 3). The answers from our reviewers about what make them identify incomparability were very diverse, in that case further investigation was conducted.

After analysis the algorithm for decision making process were drafted, which triggered by serious of morphology flags. A new purchased hematology analyzer other than primary automation is also incorporate into this process as a confirm system for blast, Van. Lym., NRBC and PLT clumps. Random revision of DC is not allowed owing to errors of variant smear preparations and limited cells counted. The most frequent causes for check by Advia were No DC and Blast(figure 1). Results of Satisfaction Survey were shown as figure 2&3.

The protocol for estimation of PLT is created through meticulous calculations and experiments, although arbitrarily using estimation of PLT to replace the instrument results is not allowable, using delta checking as criterion for replacement is encouraged.

The mean TAT, standard deviation (SD), 60th percentile, 90th percentile, and 95th percentile TAT of hematology tests were evaluated for the 263,414 specimens from Jul. 2015 to Jul. 2016. After revision algorithm implement on May. 2016, the 90th percentile TAT of hematology tests descended to less than 40 minutes(table 4), which, by t test, showed significantly different from before(p<0.0001).

Fig. 1 Frequency of causes for check by Advia

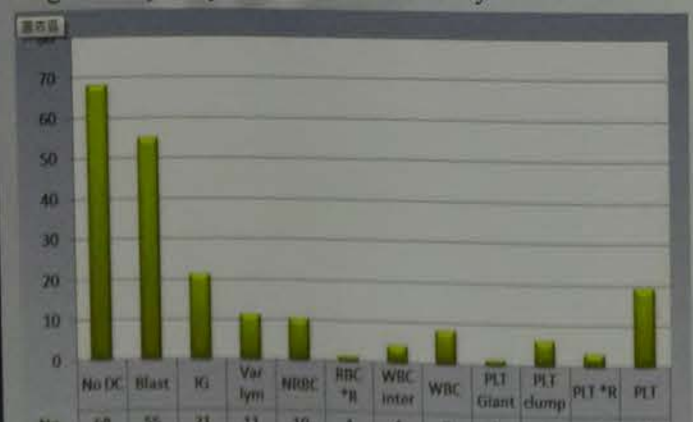


Fig. 2 Satisfaction Survey

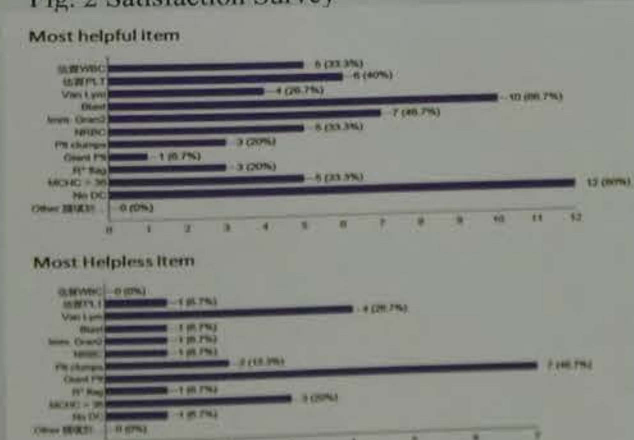
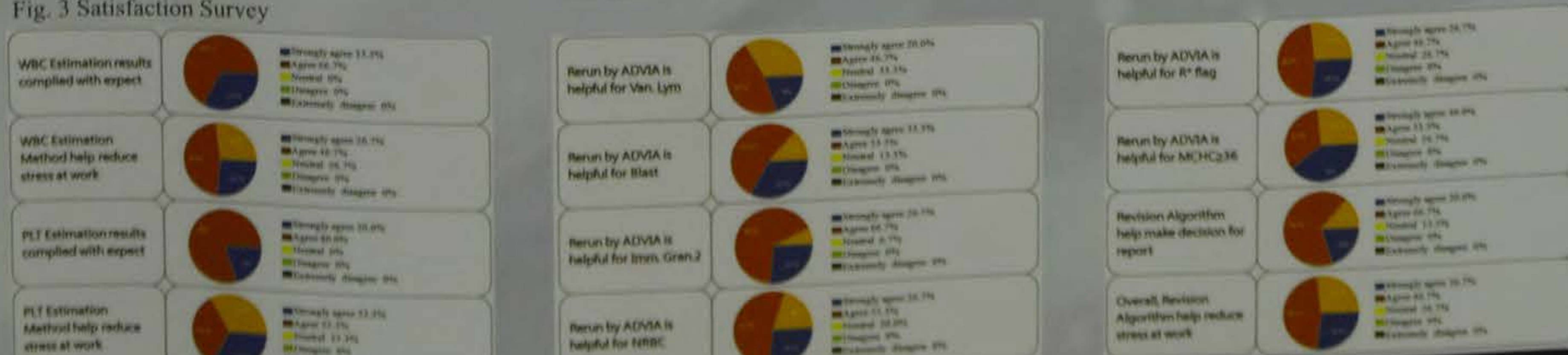


Table 4 TAT evaluation

Year_Month	Test	Mean	SD	60th percentile	90th percentile	95th percentile
2015_07	20568	17.33	21.64	11	46	61
2015_08	19915	18.92	24	13	50	67
2015_09	18647	18.36	24.74	12	46	61
2015_10	19954	19.15	22.99	15	47	61
2015_11	19817	21.73	27.28	16	56	75
2015_12	21175	18.42	20.29	15	47	60
2016_01	20935	20.03	28.35	15	49	66
2016_02	17689	18.82	22.83	14	46	65
2016_03	22412	20.94	30.87	14	53	73
2016_04	19868	18	22.2	14	46	58
2016_05	21232	17.82	23.31	12	45	62
2016_06	20281	15.96	16.92	12	39*	52
2016_07	20921	15.53	18.57	11	39*	53
total	263414				*p<0.0001	

Fig. 3 Satisfaction Survey



Conclusion

After all done, the reviewers are more clearly to understand the limitation of slide review and either comfortable to define the necessity for revisions. Overall, this revision algorithm help reduce stress at work. However some reviewers still hesitate to utilize it. In order to enhance reviewers' acknowledgement and familiarity with this algorithm, we need continue education and experience sharing.

Reference

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Hematology
PC-26

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Back to the Basic : From Platelet to Hemostasis
- The Role of Platelet in Hemostasis -

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Introduction

Platelets are important in hemostasis. When blood vessel is injured, platelets attached there as monolayer. And then platelets come in contact with each other by granules such as ADP, TXA_2 etc from alpha granule and dense core granule. Platelet is a small blood cell which is 1 to 4 micrometer and 5 to 10 days life span. It is found in bone marrow, tissue, blood vessel and shapes small discoid in resting state but change it's shape as round and further more looks like spurr cell when trigered by angonists such as collagen, TXA_2, thrombin, epinephrine, ristocetin. Platelet have two types of functional granules which is alpha and dense core granules. Alpha granule release fibrinogen, von willebrand's factor, platelet derived growth factor(PDGF), platelet factor 4(PF4), and other proteins. Dense core granule release ATP, ADP, serotonin, calcium ion and magnesium ion. Platelets have three major functions which are adhesion, granules release and aggregation.

Hemostasis is divided by primary and secondary hemostasis. During the hemostasis, platelets are concerned as primary hemostasis. Disease associated with platelets are divided by genetic disorders, genetic defects, storage pool defects, acquired defects, quantitative disorders. Bernard-Soulier disease and von willebrand's disease are genetic disorders. Glanzmann's thrombasthenia is genetic defects. Gray-platelet syndrome, Wiskott-Aldrich syndrome, Hermansky-Pudlak syndrome, Chediac-Higashi anamaly are storage pool defects. Acquired defects are caused by drug and diet. And last introduce the platelet function tests old and new.

What is Platelet

A. Thrombocyte Characteristics

1. Size : 1~4㎛
2. Reference range :
   150~400 X 10^3/㎕ (9~20/HPF)
3. Life span : 5~10 days
4. No nucleus(mature PLT)
   cytoplasm : lysosomes, mitochondria, glycogen, granules, peroxisomes
5. PLTs are found in bone marrow, tissue, blood vessels.
6. Mature PLTs are released from the bone marrow and the peripheral blood.
7. Two types of functional granules :
   1) α granules : fibrinogen, von Willebrand's factor(vWF), platelet-derived growth factor(PDGF), PF4(PLT factor), other proteins
   2) Dense core granules : ADP, ATP(energy molecules), 5-HT(serotonin), and calcium

Figure 1. Scanning electron microscope image of a red blood cell (left), platelet (center), and white blood cell (right). Credit: NCI-Frederick(http://www.daviddarling.info/encyclopedia/P/platelet.html).

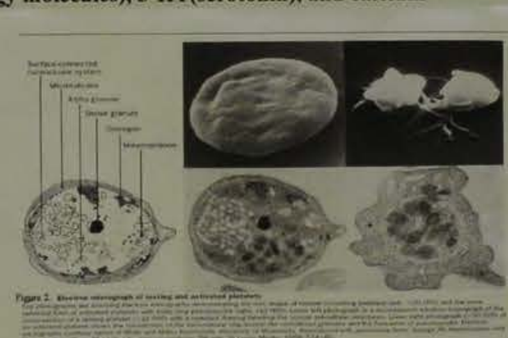

Figure 2. Electron micrograph of resting and activated platelets.

Platelet Functions

A. Adhesion

1. PLT alter their shape and adhere to vascular surface.
2. Response to subendothelial surface exposure caused by vascular injury.

B. PLT granule release

1. Regulated by :
   1) Collagen 2) Thrombin 3) Epinephrine 4) Thromboxane A_2

C. Aggregation

1. PLT clump together to form the initial plug.
2. PLT release ADP(energy source), serotonin(constricts blood vessels), PF4(neutralizes heparin).

Hemostasis overview

When blood vessel is injured :

A : At first, blood vessel is constricted at injury site and endothelial cells are exposed.

B : Platelets adhere to the injury site and form monolayers. And then platelets are changed their shape from discoid to round and spur and release granules that secret from α-granule and dense core body. This is called 'Primary Hemostasis'.

C : Platelets are activated by their granules and agonists. Then Platelets aggregated each other and hemostasis starts with coagulation factors. This is called 'Secondary Hemostasis'.

Hemostasis

A. Primary Hemostasis

1. START : PLTs contact with collagen, microfilaments, basement membrane of endothelial tissue
2. Small blood vessels constrict – PLTs adhere to exposed tissue by ADP/ATP, TXA_2
3. PLTs begin to aggregation – release ADP, ATP, serotonin

B. Secondary Hemostasis

1. Formation of a fibrin clot - intrinsic and extrinsic coagulation pathways.
2. Fibrin clot includes fibrin formed (secondary hemostasis) and the PLT plug formed (primary hemostasis)
3. Intrinsic pathway – specific coagulation proteins come in contact with subendothelial tissue
4. Extrinsic pathway – starts with the release of tissue factor from injured blood vessel endothelial cells and subendothelium.
5. Common pathway – begins with factor X activation
6. Alternative pathway – link the extrinsic, intrinsic and common pathways.

Platelet Function Tests

Laboratory tests of platelet function are traditionally utilized for diagnosing hemostasis diseases such as bleeding time, light transmission aggregation, lumiaggregometry, impedance aggregation on whole blood, and flow cytometry. There are some pors and cons opinions of platelet function tests from traditional bleeding time to POCT(table 1). To date, platelet function tests have developed with easy and rapid methods and expected more POCT than any other tests due to diagnosis related platelet disorders.

Table 1 Pros and Cons of reviewed platelet function tests

Method	Pro	Contra
Bleeding time	In vivo test, quick No WB processing	Invasive Poorly standardized Dependent on many variables (skin thickness, and temperature operator's skills)
Light transmission platelet aggregation (LTA)	Historical gold standard Diagnostic method Flexible Different platelet pathways investigated Sensitive to anti-plt therapy	Manual sample processing Pre- and analytic variables High sample volume Time-consuming
Impedance Platelet Aggregometry	No sample processing Diagnostic method Flexible Different platelet pathways investigated Sensitive to anti-plt therapy Close to POCT (multiple system)	Limited HCT and platelet count range
VerifyNow system	Waived POCT No WB processing Easy, quick	Nonflexible Very expensive Monitoring antiplatelet therapy Limited HCT and platelet count Indirect assay Required adjunctive platelet count
Plateletworks	POC system Minimal sample preparation Easy, rapid screening test	Scarce data
PFA-100, Innovance PFA-200	In vitro standardized BT Easy, quick POCT Sensitive to severe platelet defects	Rigid closed system Platelet count – HCT dependent Not sensitive to platelet secretion defects
Impact Cone and Plate(let) Analyzer	WB assay Global platelet method Small sample volume	Expensive Experienced staff Lack of clinical studies Not widely available
Global thrombosis test (GTT)	WB assay Global platelet method Small sample volume POCT	Lack of clinical studies Not widely available
TEG platelet mapping	Global hemostasis test Reduces blood transfusions POCT	Measure clot properties More studies are needed
ROTEM platelet	Predicts bleeding Reduces blood transfusions Improves clinical outcome Global hemostasis test WB platelet aggregometry	Limited HCT and platelet count range (for platelet system) Lack of clinical studies

Abbreviations: BT, bleeding time; HCT, hematocrit; [illegible] POCT, point-of-care testing; ROTEM, rotational thromboelastometry; [illegible] WB, whole blood. (Cited by Platelet function tests: a comparative review. Vascular Health and Risk Management 2015:11 133-148)

Hematology

PC-27

Effects of Improperly-Filling Dipotassiumtetraacetic Acid (K_2EDTA) with Blood of Clinically Diagnosed Dengue and Non Dengue Individuals

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ABSTRACT

Under filling and overfilling K_2EDTA tubes is an inevitable practice in hospitals especially in wards where patients have fragile veins and are considered hard-to-extract patients. The researchers looked into the hematologic effects of improperly filling K_2EDTA tubes in the complete blood count of clinically diagnosed dengue patients and healthy individuals. A total sample size of 48 consisting of 24 individuals with ages ranging from seven to 45 years old, diagnosed with dengue fever syndrome, and 24 healthy individuals with the same age range were selected for the study. Six milliliters of venous blood were put on three tubes by under filling, properly filling, and overfilling each tube; and ran in time intervals of 30 minutes, one, two, and four hours. Using SPSS 20.0, the parameters affected by improperly filling the tubes were RBC (p-value=0.027), platelet (p-value=0.035), and segmenters (p-value =0.011); by time of feeding were hemoglobin (p-value =<0.001), hematocrit (p-value =0.034), segmenters (p-value=<0.001), lymphocytes (p-value=<0.001), monocytes (p-value=0.001), and eosinophils (p-value=0.006); by control group were WBC (p-value=<0.001), hemoglobin (p-value=0.018), hematocrit (p-value=0.008), and platelet (p-value=<0.001); by volume and time was hematocrit (p-value=0.042); by control group and time were monocytes (p-value=0.0030), and basophils (p-value=0.001).

KEYWORDS: Complete Blood Count, Dengue, EDTA

INTRODUCTION

Benachinmardi et al. (2013) stated that hematological examination is an important step in managing dengue patients. The International Council for Standardization in Hematology currently recommends ethylenediaminetetraacetic acid as the anticoagulant of choice for the hematological testing. It is best for preserving cellular components and morphology of blood cells (Lippi et al., 2007). Delayed sample analysis could result in hematological changes in the measured parameter, which could complicate the interpretation of the resulting data. This is likely to result in wrong reporting and subsequently wrong diagnoses (Antwi-Baffour et al., 2013).

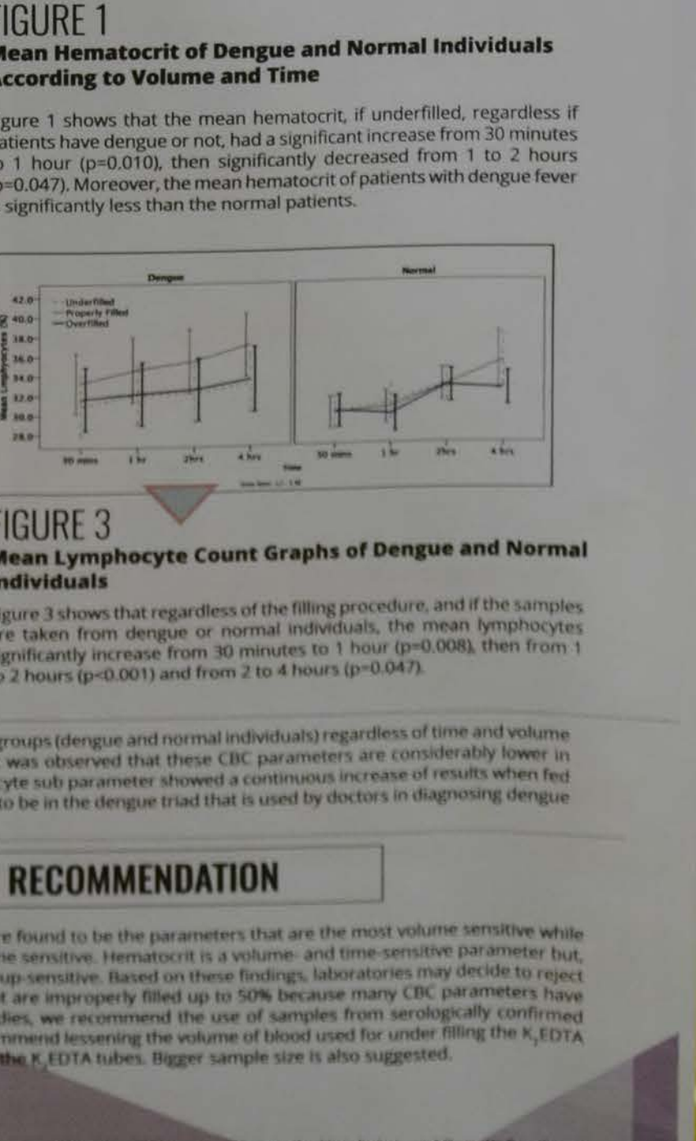
OBJECTIVES

The objective of this research was to determine the hematological effects of improperly filled K_2EDTA tubes (underfilled and overfilled) in the complete blood count of dengue and normal individuals.

SPECIFIC OBJECTIVES

- Dengue and normal patients: To determine significant changes in CBC parameters of blood in EDTA tubes that were filled up in three different ways in varying time interval:
  - **01** Properly filled (control)
  - **02** Improperly filled (experimental)
    - A. Underfilled
    - B. Overfilled
- To determine if there was a significant change in various percentage differences of CBC parameters observed overtime

METHODOLOGY



RESULTS AND DISCUSSION

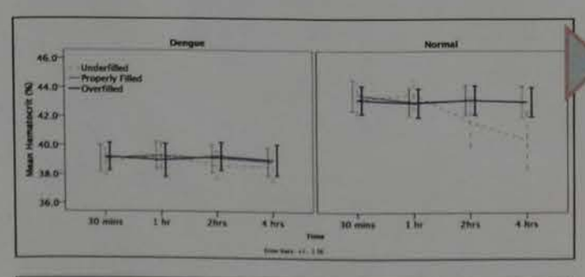


FIGURE 1

Mean Hematocrit of Dengue and Normal Individuals According to Volume and Time

Figure 1 shows that the mean hematocrit, if underfilled, regardless if patients have dengue or not, had a significant increase from 30 minutes to 1 hour (p=0.010), then significantly decreased from 1 to 2 hours (p=0.047). Moreover, the mean hematocrit of patients with dengue fever is significantly less than the normal patients.

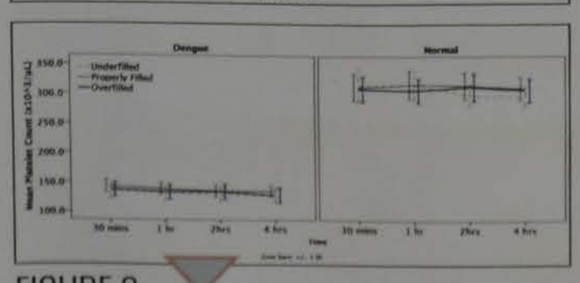


FIGURE 2

Mean Platelet Count of Dengue and Normal Individuals

Figure 2 shows that for the filling procedure, regardless if patients have dengue or not, if underfilled (p=0.038), the mean platelet counts are significantly less than the properly filled. The mean platelet counts of properly filled and overfilled did not differ (p=0.080).

FIGURE 3

Mean Lymphocyte Count Graphs of Dengue and Normal Individuals

Figure 3 shows that regardless of the filling procedure, and if the samples are taken from dengue or normal individuals, the mean lymphocytes significantly increase from 30 minutes to 1 hour (p=0.008), then from 1 to 2 hours (p<0.001) and from 2 to 4 hours (p=0.047).

Significant changes that were observed when comparing the groups (dengue and normal individuals) regardless of time and volume were seen in the hematocrit (Hct), and platelet (Plt) count. It was observed that these CBC parameters are considerably lower in dengue patients than in normal individuals. Only the lymphocyte sub parameter showed a continuous increase of results when fed at different time intervals. These parameters are considered to be in the dengue triad that is used by doctors in diagnosing dengue fever.

CONCLUSION AND RECOMMENDATION

The RBC count, platelet count, segmenters and monocytes were found to be the parameters that are the most volume sensitive while hemoglobin, segmenters, lymphocytes, and eosinophils are time sensitive. Hematocrit is a volume- and time-sensitive parameter but, on the other hand, monocytes and basophils are time- and group-sensitive. Based on these findings, laboratories may decide to reject specimens that are collected after 2 hours and specimens that are improperly filled up to 50% because many CBC parameters have proven to be unstable in these circumstances. For future studies, we recommend the use of samples from serologically confirmed dengue patients and other pathological samples. We also recommend lessening the volume of blood used for under filling the K_2EDTA tubes and increasing the amount of blood used for over filling the K_2EDTA tubes. Bigger sample size is also suggested.

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Hematology
PC-28

Plasma Hemoglobin versus Haptoglobin and The Others Parameters

The Discussion of Intravascular Hemolysis Pattern

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Introduction

While increasing destruction of RBC in intravascular hemolysis, the level of free hemoglobin in plasma (plasma-Hb, αβ dimers) shall be elevated and integrate with haptoglobin. Plasma haptoglobin level was depleted in the presence of large amount of liberated free hemoglobin. Then the hemoglobin-haptoglobin complexes were removed by macrophages in the reticuloendothelial system like spleen, liver and lymph nodes. Besides, the excess amount of plasma-Hb (αβ dimers form) would filter through the glomerulars and shall be reabsorbed in proximal tubule cells, if not, the hemoglobinuria would present. Hemosiderin would be found in urine 3-4 days after the onset of hemolysis and persist in several weeks.

Clinically, we determined the hemolytic anemia via physical examination and clinical tests. If each clinical test is used alone, the specificity is not high enough for diagnosis and could not exactly evaluate patients' condition. In addition to the biochemical and CBC tests, plasma hemoglobin and urine hemosiderin would assist in diagnosis and evaluating the efficacy of treatment in house laboratory. Several markers have been described in multiple researches and altered in variable hemolytic conditions. In this study, we focus on analysis of the relationship between plasma hemoglobin and other hemolytic markers such as haptoglobin, urine hemosiderin and, LDH. Besides, we concerning whether the combination of LDH, haptoglobin, and plasma hemoglobin would improve the specificity of hemolytic anemia and differentiate the expression level of hemolytic markers of intravascular from extravascular hemolysis

Materials & Methods

Patient population

The patient population consisted of 69 random cases with suspected hemolytic anemia (simultaneously comprising values of haptoglobin and plasma hemoglobin) since August, 2014. 33 patients with an established diagnosis of hemolytic anemia and were recruited in this study in division of Hematology and Oncology at the Taipei Veterans General Hospital. These patients were divided into three groups (group I/II/III) based on plasma hemoglobin level.

Sample collection

The hemolytic plasma specimens would be rejected in our collection for plasma hemoglobin quantitation. The detection of plasma hemoglobin was performed by tetramethylbenzidine (TMB)-colorimetry.

Statistical analysis

Analysis of the relationship between plasma hemoglobin and other hemolytic markers performed with simple linear regression and observed in scatter diagram. The levels of LDH expression among three groups were analyzed by t-test.

Results

There were high sensitivity and low specificity when each test was performed alone. Although the sensitivity of three tests combination of LDH, haptoglobin and plasma hemoglobin would not be increased, the specificity is greater than each test performed alone and even greatly elevated up to 71%. (Table. 1)

LDH	Hemolytic anemia	non-Hemolytic anemia
>250 U/L	66%	58%
131-250 U/L	34%	42%

Haptoglobin	Hemolytic anemia	non-Hemolytic anemia
<30 mg/dL	91%	57%
30-200 mg/dL	9%	43%

Plasma HGB	Hemolytic anemia	non-Hemolytic anemia
>5 mg/dL	79%	56%
≦5 mg/dL	21%	44%

LDH Haptoglobin Plasma HGB	Hemolytic anemia	non-Hemolytic anemia
All the above tests matched	62%	29%
Either one of the tests mismatched	38%	71%

Table. 1 The sensitivity and the specificity of hemolytic-associated tests and different combination. There were 69 cases analysis both plasma hemoglobin and haptoglobin tests, among with 33 diagnosed hemolytic anemia and 36 non-hemolytic anemia. There were 53 cases analysis LDH tests, among with 29 diagnosed hemolytic anemia and 24 non-hemolytic anemia. Thus, there were total 53 cases analysis all three test.

The reference range of plasma hemoglobin concentration is ≦ 5 mg/dL in house laboratory and, in clinical, the concentration >20 mg/dL result in hemoglobinemia and hemoglobinuria. Thus, we were interested in how the hemolytic markers change when the concentration of plasma hemoglobin was divided into three groups: ≦5 mg/dL, 5-20 mg/dL and >20mg/dL according to clinical significance. To realize whether the hemolytic-associated markers changed with the concentration of plasma hemoglobin increased, p-value between each test were calculated by t-tests.

According to the simple linear regression model, we found that moderate positive-correlation (R2= 0.747) existed in between LDH and plasma hemoglobin. (Figure. 1A) The LDH level was elevated with the concentration of plasma hemoglobin increased (Figure. 1B).

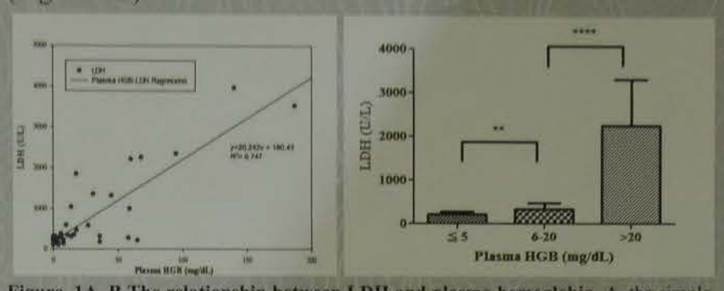

Figure. 1A, B The relationship between LDH and plasma hemoglobin. A, the simple linear regression of LDH on plasma hemoglobin; B. Variation of LDH level in three groups by t-test analysis. The p-value< 0.01(**) and p-value< 0.0001 (****) were indicated.

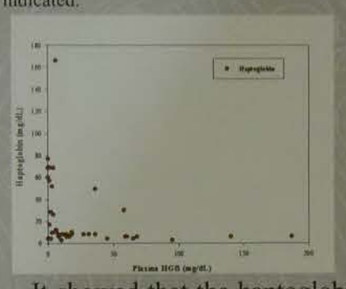

Figure. 2 The relationship between haptoglobin and plasma hemoglobin. The scatter diagram of haptoglobin on plasma hemoglobin.

It showed that the haptoglobin level was weakly correlated to plasma hemoglobin level in scatter diagram. When most of the plasma hemoglobin level ≦5 mg/dL, haptoglobin results would be within reference range in the same patient. Otherwise, the haptoglobin level was depleted in the plasma hemoglobin level >5 mg/dL. The haptoglobin level would reflect when hemolysis occurred, but could not help monitor recovery from hemolysis or response to therapy. Besides, haptoglobin level was reduced in other condition such as impair liver function, malnutrition and congenital hypohaptoglobinemia, which influenced its specificity, so it would not enough to be a great indicator for monitoring in treatment phase. Then, changes of plasma hemoglobin would help provide the degree of different hemolysis for clinical treatment.

Table. 2 Hemolytic markers between intravascular and extravascular.

	Hemolysis						
	Intravascular			Extravascular			Uncertain
	PNH	TMA	Intravascular devices/Valve disorders	AIHA	Membrane/Enzyme defects	CDA (Thalassemia)	
Total (Case numbers)	4	0	5	10	4	5	5
Plasma Hemoglobin (Increased)	3	N/A	5	6	3	5	5 (n= 4)
LDH (Increased)	3	N/A	5	7	0	2 (n=4)	5
Haptoglobin (Decreased)	3	N/A	5	10	4	5	5
Urine Hemosiderin (Present)	3 (n= 3)	N/A	5	7 (n= 9)	1 (n= 1)	4	3 (n= 3)
Fragmented RBC (Present)	4	N/A	5	5 (n= 5)	0 (n= 2)	3 (n= 3)	2 (n= 4)

PNH, paroxysmal nocturnal hemoglobinuria; AIHA, autoimmune hemolytic anemia; CDA, congenital dyserythropoietic anemia, TMA, thrombotic microangiopathies

The hemolytic markers would apparently reflect the hemolysis in intravascular, especially LDH and plasma hemoglobin. The levels of LDH and plasma hemoglobin in intravascular were higher than in extravascular and the statistical significance were attained. The haptoglobin level would not differentiate intravascular from extravascular when severe hemolysis occurred. (Figure. 3)

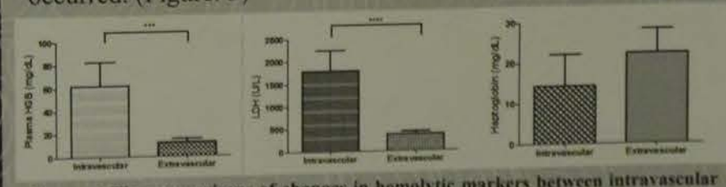

Figure. 3 The comparisons of changes in hemolytic markers between intravascular and extravascular hemolysis. The p-value< 0.001(***) and p-value< 0.0001 (****) were indicated.

Discussion

To investigate a case of paroxysmal nocturnal hemoglobinuria in a male whose report of RBC count, Hb, MCV, bilirubin, haptoglobin and plasma Hb were normal as usual. The report of only urine hemosidrin could reflect the hemolytic condition of patient. There is not any test could absolutely monitor the hemolysis when performed alone, so we suggested that urine hemosiderin should be added into order by doctor.

Conclusion

In our analysts, we assessed the plasma hemoglobin levels in predicting clinical outcome for monitoring recovery from hemolysis. The plasma hemoglobin is a greater indicator than haptoglobin when hemolytic patients were in treatment phase. Then, we also found that the combination of three hemolytic-associated tests would elevate the specificity of hemolytic anemia.

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Hematology
PC-29

Back to the Basic : From Platelet to
- The Role of Platelet in Hemostas

Hyo Chan Kang

Department of Medical Laboratory Science, Dongeui Institute of Tech

Hematology

PC-31

Expression and localization of RNase and RNase inhibitor in blood cells

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Introductions

Extracellular RNA may be released from cells in cases of injury and vascular disease. It has been recognized as a novel procoagulatory and permeability-increasing factor and is counteracted by RNase. No detailed characterization of the expression and localization of RNase and its inhibitor (RI) in the vasculature has been carried out so far.

We aimed to investigate the expression and localization of RNase and RI in cells which come in contact with blood, such as platelets, mononuclear cells (MNCs), polymorphonuclear cells (PMNs), and red blood cells (RBCs) compared with human umbilical vein endothelial cell line EAhy926 cells. We further investigated the effect of thrombin on the expression of RNase 1 and RI in platelets.

Materials & Methods

Materials

A) Reagents
- thrombin : 2 nM

B) Cells
- EAhy926 (human umbilical vein endothelial cell line)
- platelets
- mononuclear cells (MNCs)
- polymorphonuclear cells (PMNs)
- red blood cells (RBCs)

Methods
- RNase activity tests
- reverse transcription-polymerase chain reaction (RT-PCR)
- western blot
- immunocytochemical staining
- transmission electron microscopy (TEM)
- immunoelectron microscopy (pre-embedding method, post-embedding method)

Conclusions

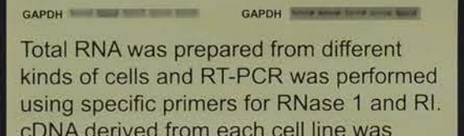
- RNase 1 activity, mRNA and protein expression derived from EAhy926 were highest, but RI mRNA and protein were similarly expressed in most of the cells.
- RNase activity may be mainly released by vascular endothelial cells in the vasculature, and RNase 1 released from blood cells seems to be inhibited by RI.
- RNase 1 and VWF were partly colocalized in EAhy926 Weibel-Palade bodies and platelet a-granules.

Healthy vascular endothelial cells support anticoagulant property by secreting RNase activity, but at the sites of endothelial injury, RNase activity may be blocked by platelets and leukocytes. The RNA/RNase 1/RI in blood cells may be a contributor to the regulation and maintenance of vascular homeostasis.

Results

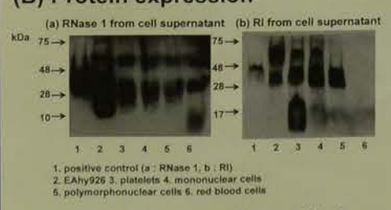
Fig 1. RNase 1 and RNase inhibitor (RI) expression in blood cells.

(A) mRNA expression

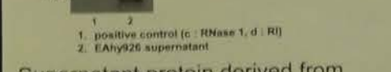


Total RNA was prepared from different kinds of cells and RT-PCR was performed using specific primers for RNase 1 and RI. cDNA derived from each cell line was amplified by 30 cycles.
GAPDH mRNA was used as a mRNA quantity control.
Each value represents the mean ± SD (n = 6) compared to EAhy926.
Significant differences are indicated (**) with ($p < 0.01$), whereas (*) indicates a significant difference with ($p < 0.05$).

(B) Protein expression



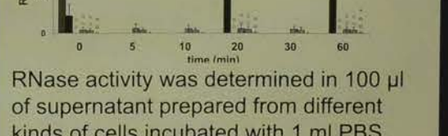
After prepared from different kinds of cells, supernatant was prepared and used for western blot analysis using antibodies against RNase 1 (poly) and RI (mono).



Supernatant protein derived from EAhy926 was dissociated in 8 M guanidinium chloride (a very strong chaotropic agent) and used for western blot analysis using antibodies against RNase 1 (poly) and RI (mono).

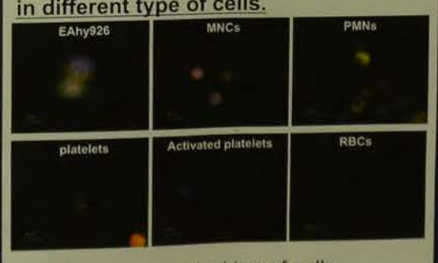
Fig 2. RNase activity

RNase activity in supernatant derived from EAhy926 cells and blood cells incubated for different times.



RNase activity was determined in 100 µl of supernatant prepared from different kinds of cells incubated with 1 ml PBS. Each value represents the mean ± SD (n = 6).
Significant differences are indicated (○○ or **) with ($p < 0.01$) compared to EAhy926 in cell types or 0 min values of each cells, respectively, whereas (*) indicates a significant difference with ($p < 0.05$).
Activity values of platelets were normalized to the same cell number (1 x 10^8 cells), the others were 1 x 10^6 cells.

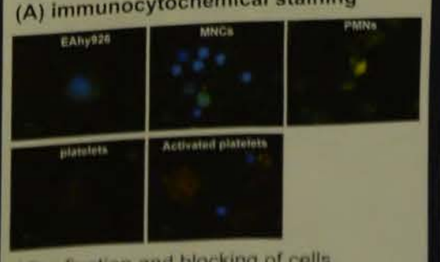
Fig 3. Localization of RNase 1 and RI in different type of cells.



After fixation and blocking of cells, antibodies against RNase 1 (green) and RI (red) were added followed by application of the corresponding secondary antibodies and Hoechst-staining of the nuclei (blue). Scale bar equals 10 µm.

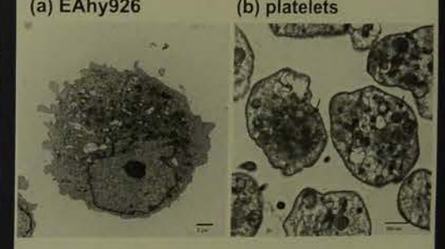
Fig 4. Localization of RNase 1 and von Willebrand factor (VWF) in different type of cells.

(A) immunocytochemical staining



After fixation and blocking of cells, antibodies against RNase 1 (green) and VWF (red) were added followed by application of the corresponding secondary antibodies and Hoechst-staining of the nuclei (blue). Scale bar equals 10 µm.

(B) transmission electron microscopy (TEM)



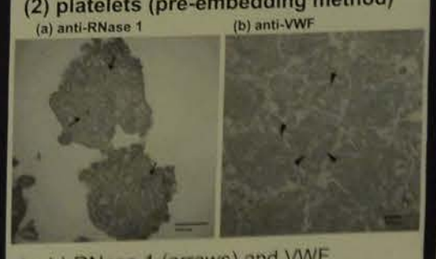
(a) TEM revealed EAhy926 cells. (magnification, x5000)
(b) TEM revealed α-granules (arrows) of platelets. (magnification, x30000)

(C) immunoelectron microscopy

(1) EAhy926 (pre-embedding method)

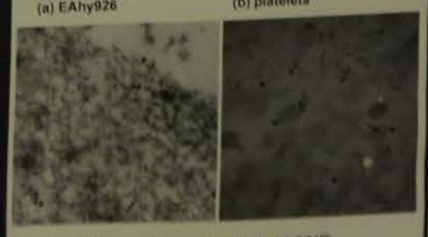
(a, b) RNase 1 (arrows) and VWF (arrowheads) were localized in EAhy926. But they were not in nuclei (N). (magnification, x40000, x30000)

(2) platelets (pre-embedding method)



(a, b) RNase 1 (arrows) and VWF (arrowheads) were partly colocalized in α-granules. (magnification, x40000, x20000)

(3) post-embedding method



(a, b) RNase 1 (arrows) and VWF (arrowheads) were partly colocalized in EAhy926 and α-granules. (magnification, x80000, x80000)

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The 32nd World Congress of Biomedical Laboratory Science

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TOKYO MEDICAL AND DENTAL UNIVERSITY TMDU

Hematology

PC-32

Cell-based laboratory evaluation of coagulation activity change by antineoplastic drugs for the treatment of hematological tumors

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Introduction

Idarubicin (IDR), doxorubicin (DXR), and vorinostat (VOR) are effective for treatment of myeloid and lymphoid tumors. During the initial course of induction therapies activation of intravascular coagulant activity is induced, which leads to disseminated intravascular coagulation (DIC) or deep vein thrombosis (DVT).

We examined the procoagulant effects of IDR, DXR, and VOR *in vitro*, especially focusing on tissue factor (TF) and anionic phospholipid phosphatidylserine (PS).

Materials

A) reagents (of pharmacological concentration)
- idarubicin (IDR) : 0.02 μM, 0.2 μM
- doxorubicin (DXR) : 0.1 μM , 1.0 μM
- vorinostat (VOR) : 0.5 μM, 1.0 μM, 2.0 μM

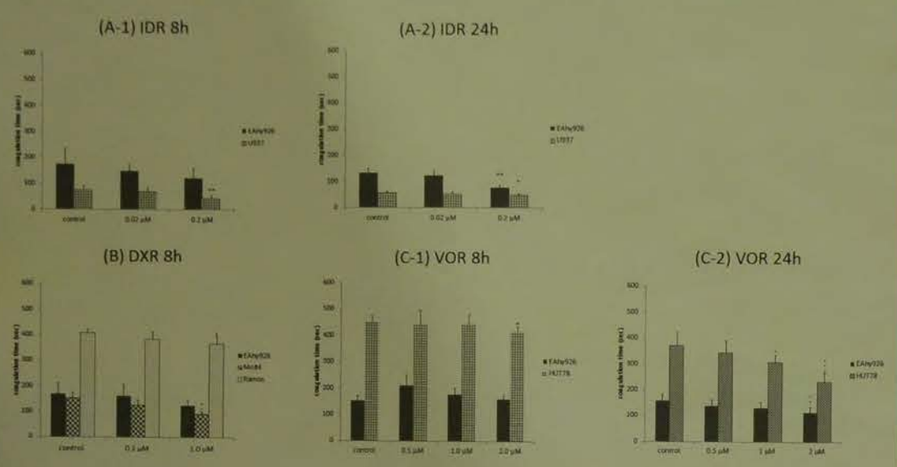
B) cell lines (2 x 10^6 cells)
- EAhy926 (umbilical vein endothelial)
- U937 (acute monocytic leukemia)
- Molt4 (acute T-lymphoblastic leukemia)
- Ramos (Burkitt (B cell) lymphoma)
- HUT78 (cutaneous T cell lymphoma)

Methods

(1) procoagulant activity (PCA)
→*normal plasma-based recalcification time assay*
(2) cell surface tissue factor (TF) antigen
→*flow cytometric analysis*
(3) quantification of TF mRNA
→*RT-PCR*
(4) cell surface phosphatidyl serine (PS) exposure
→*flow cytometric analysis*

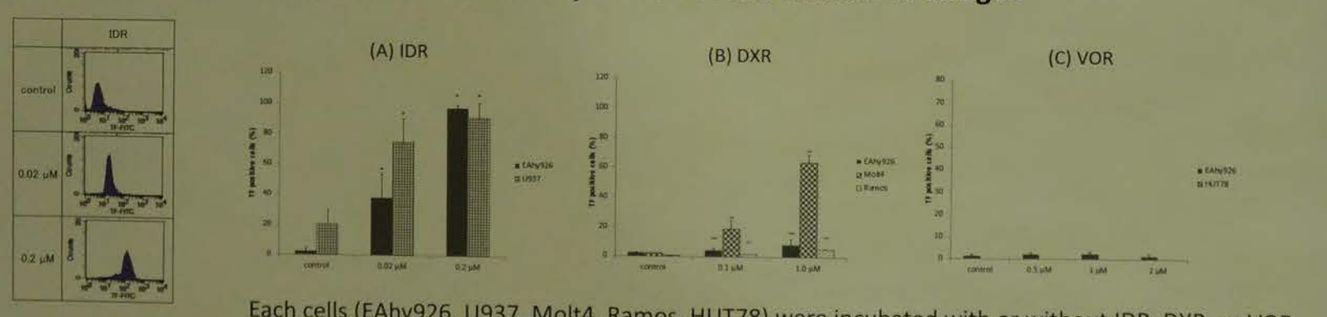
Result

Fig 1. Effects of IDR, DXR, or VOR on cell surface PCA



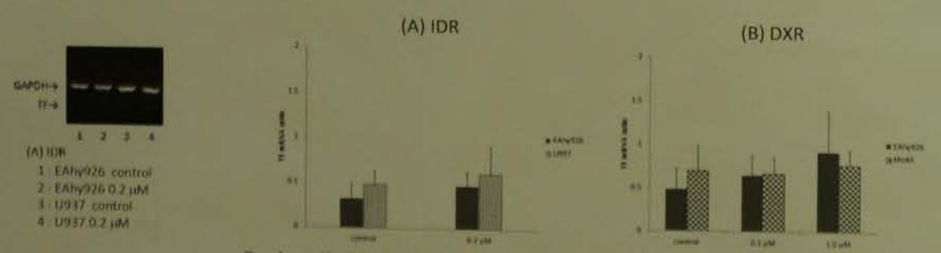
2 x 10^6 of each cells (EAhy926, U937, Molt4, Ramos, HUT78) were incubated with or without IDR, DXR, or VOR, respectively, at 37°C for 8 h or 24 h. Data are expressed as mean ± SD (n = 6). Significant differences are indicated (*) with ($p < 0.05$) , whereas (**) indicates a significant difference with ($p<0.01$), and (***) with ($p<0.001$) compared to control.

Fig 2. Effects of IDR, DXR, and VOR on the expression of cell surface TF antigen



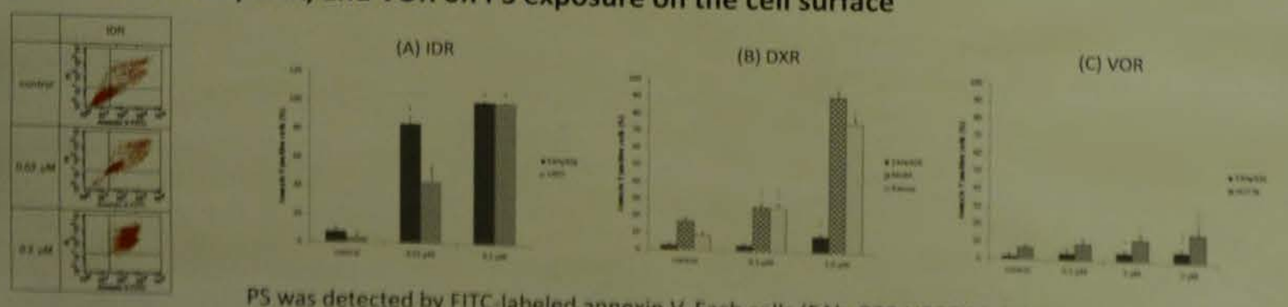
Each cells (EAhy926, U937, Molt4, Ramos, HUT78) were incubated with or without IDR, DXR, or VOR, respectively, at 37°C for 24 h. Data are expressed as mean ± SD (n = 6).

Fig 3. Effects of IDR and DXR on TF mRNA



Each cells (EAhy926, U937, Molt4) were incubated at 37°C for 4 h. Total RNA was extracted from and stocked until RT-PCR. cDNA derived from each cell line was amplified by 28 cycles. GAPDH mRNA was used as an mRNA quantity control. Relative signal intensity was determined and data are expressed as mean ± SD (n = 5).

Fig 4. Effects of IDR, DXR, and VOR on PS exposure on the cell surface



PS was detected by FITC-labeled annexin V. Each cells (EAhy926,U937,Molt4, Ramos, HUT78) were incubated with or without, IDR, DXR or VOR at 37°C for 24 h. Data are expressed as mean ± SD (n = 6).

Conclusions

IDR and DXR induced PCA on vascular endothelial and myeloid lymphoid tumor cells through cell surface TF antigen and PS exposure, while TF mRNA was not induced.

VOR induced PCA on vascular endothelial and lymphoid tumor cells through PS exposure.

Cell-based laboratory methods presented in this study may be useful for evaluating procoagulant effects of antineoplastic drugs.

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Hematology
PC-33

Classification of acute promyelocytic leukemia by setting high myeloperoxidase activity area in ADVIA cytogram

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2) Kobe Univ. Graduate School of Health Sciences

Introduction

- Several studies on the classification of acute myeloid leukemia (AML) using hematological analyzers have been performed.
- Strong positivity of myeloperoxidase represents the typical cytochemical pattern of acute promyelocytic leukemia (APL) cells, and it is empirically known that APL cases show typical cytogram pattern in ADVIA PEROX channel.
- The aims of this study were to distinguish APL from other AML subtypes and to detect morbid neutrophils remaining during the routine classification of blood cells by setting the high myeloperoxidase activity area in PEROX cytogram.
- The significant reduction in the number of cells in setting area for APL patients was observed during their progress to complete remission.
- The differentiation-induced morbid cells remaining during the treatment period could be detected by setting area at cytogram, although microscopy couldn't detect morbid cells maintaining strong peroxidase activity.

Methods

- We studied medical records of 5 patients with APL and 15 patients with other AML subtypes and analyzed the frequency of cells on the high myeloperoxidase activity area using the FCS system (Siemens). Healthy volunteers were enrolled as controls.
- We also investigated changes in the number of cells in setting area for APL patients during their progress to complete remission.
- Statistical comparisons between groups were performed using a one-way ANOVA followed by the Tukey's multiple comparison test.

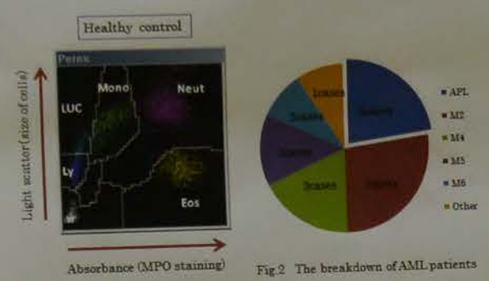


Fig.1 ADVIA2120i PEROX cytogram

Fig.2 The breakdown of AML patients

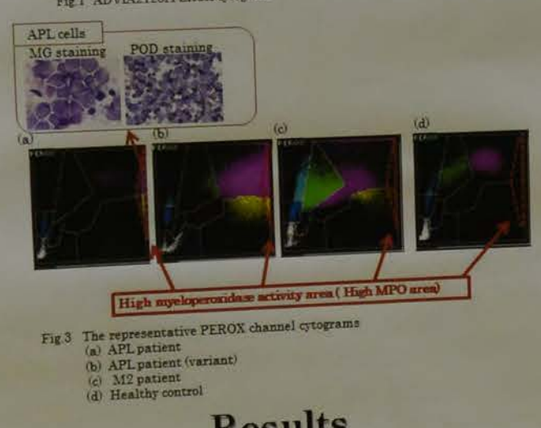


Fig.3 The representative PEROX channel cytograms
(a) APL patient
(b) APL patient (variant)
(c) M2 patient
(d) Healthy control

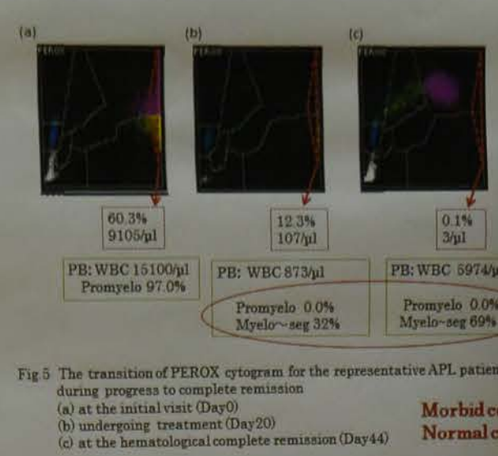


Fig.5 The transition of PEROX cytogram for the representative APL patient during progress to complete remission
(a) at the initial visit (Day0)
(b) undergoing treatment (Day20)
(c) at the hematological complete remission (Day44)

The transition of differential white blood count

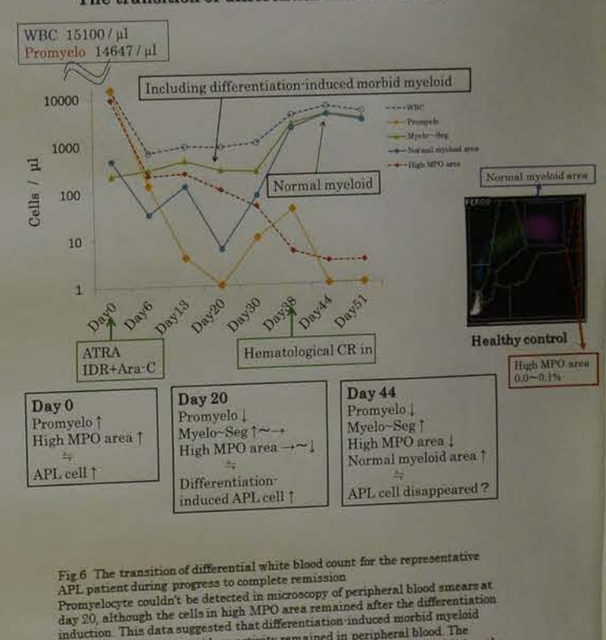


Fig.6 The transition of differential white blood count for the representative APL patient during progress to complete remission
Promyelocyte couldn't be detected in microscopy of peripheral blood smears at day 20, although the cells in high MPO area remained after the differentiation induction. This data suggested that differentiation-induced morbid myeloid maintaining strong peroxidase activity remained in peripheral blood. The number of cells in high MPO area was normalized after Day 44. In addition, the number of cells in normal myeloid area was correlated with the number of neutrophils in microscopy of peripheral blood smears after Day44.

Results

- The frequencies of the cells in the setting area for APL patients were significantly higher than those for patients with other AML subtypes and for the healthy controls.

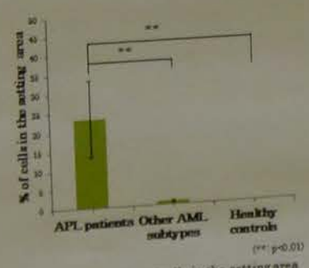


Fig.4 The frequencies of cells in the setting area
Each data point represents the average of each group; bars, ± SE.

Conclusions

- These data suggest that information from the cytogram obtained by using the hematological analyzer ADVIA could be useful for the classification of APL and for the detection of morbid cells remaining during the treatment period.

Hematology
PC-34

Significance of [illegible] diagnosis of APL and other

Takako Tenjin1, Kenji Yonezawa1, Chika Omoto1, Tomoe [illegible]
Masanori Nakamura1, Toru Murayama1, Mitsuhiro Ito2
1 Hyogo Cancer Center, Japan
2 Kobe Univ. Graduate School of Health Sciences

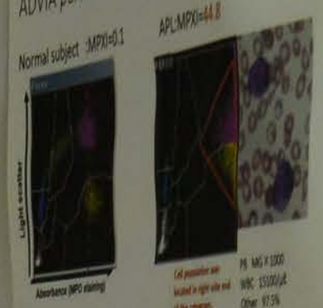
Introduction

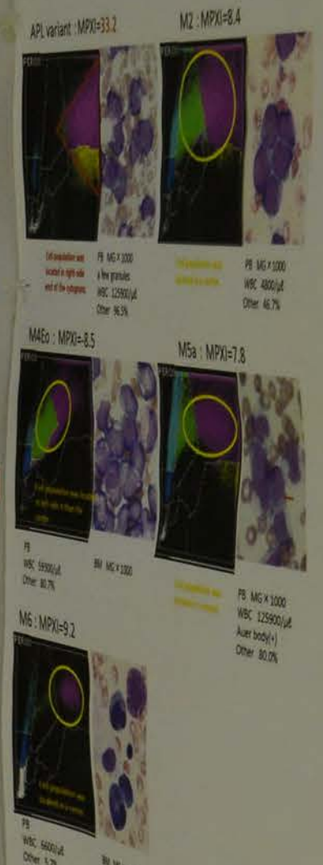
- The mean myeloperoxidase index (MPXI) is calculated during the routine complete blood count (CBC) performed using the autoanalyzer ADVIA2120i.
- We previously reported that the MPXI in acute promyelocytic leukemia (APL) patients was higher than that in patients with the other types of AML. Furthermore, MPXI was useful in the follow-up of APL.
- In this study, we studied a larger samples size and provided statistical reports showing that the significance of the MPXI in more detail.

Materials and Methods①

- Statistical analyses were performed using ANOVA and Tukey's tests. The significance for each test was set at P values less than
- All statistical analyses were performed using EZR, which is a graphical user interface

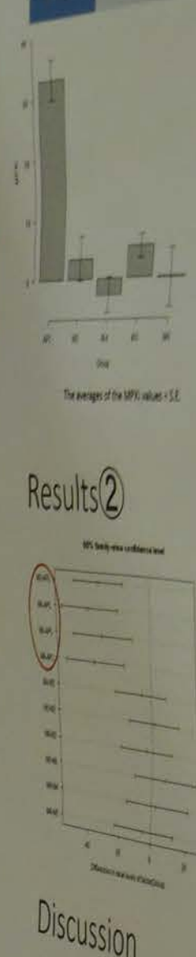
ADVIA peroxidase cytograms and microgram





Results①

The averages of the MPXI values in the groups were as follows.



- The P value
- The difference

Results②

The differ
average M
between
and the c
groups w
significan

Discussion

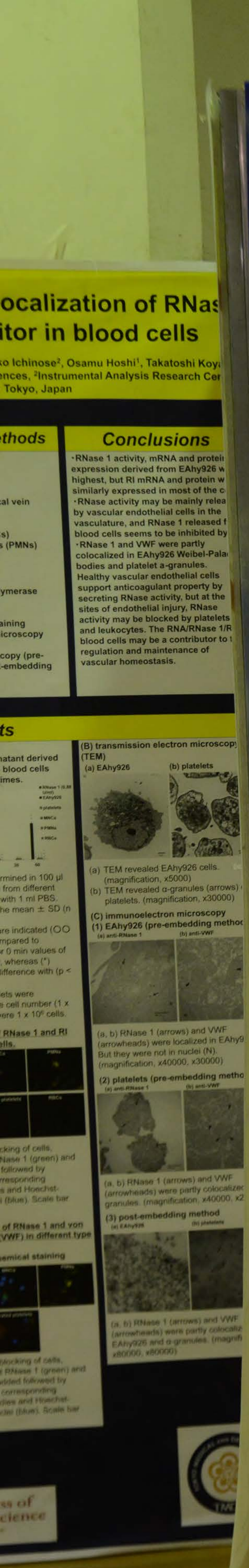
- These data suggest that MPXI values for the diagnosis of APL or the other subtypes. Interestingly, MPXI is effec differential diagnosis in the case that to diagnose morphologically including variant and the M2.
- It was thought that the remarkable hi MPXI reflected abnormal granule (MP positive granule) of APL.

Conclusion

- We measured the MPXI in patients w using ADVIA2120i.
- The MPXI in APL patients was higher statistical significance than that in pa the other types of AML.
- MPXI values are useful for the diagn subtypes.

Materials and Methods①

- We measured the MPXI in 26 patients with AML treated at the Hyogo Cancer Center using an appropriately controlled ADVIA2120i.



Hematology

PC-34

Significance of the MPXI in the differential diagnosis of APL and other subtypes of AML

Takako Tenjin1, Kenji Yonezawa2, Chika Omoto1, Tomoe Yagi1, Aya Adachi1, Masanori Nakamura1,Toru Murayama1, Mitsuhiro Ito3

1 Hyogo Cancer Center, Japan

2 Hyogo Cancer Center/Kobe Univ.Greduate School of Health Sciences

3 Kobe Univ.Greduate School of Health Sciences

Introduction

- The mean myeloperoxidase index (MPXI) is calculated during the routine complete blood count (CBC) performed using the autoanalyzer ADVIA2120i.
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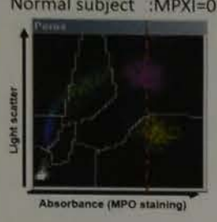
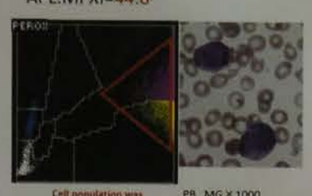
- In this study, we studied a larger samples size and provided statistical reports showing that the significance of the MPXI in more detail .

ADVIA peroxidase cytograms and micrograms

Normal subject :MPXI=0.1



APL:MPXI=44.8

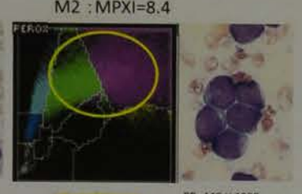


PB MG×1000
WBC 15100/μℓ
Other 97.5%

APL variant : MPXI=33.2

PB MG×1000
a few granules
WBC 125900/μℓ
Other 96.5%

M2 : MPXI=8.4



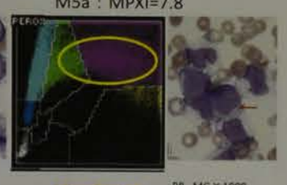
PB MG×1000
WBC 4800/μℓ
Other 46.7%

M4Eo : MPXI=-8.5

PB
WBC 59300/μℓ
Other 80.7%

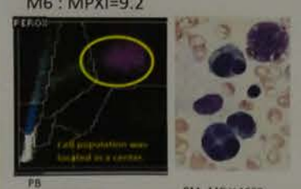
BM MG×1000

M5a : MPXI=7.8



PB MG×1000
WBC 125900/μℓ
Auer body(+)
Other 80.0%

M6 : MPXI=9.2

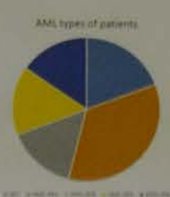


PB
WBC 6600/μℓ
Other 5.7%

BM MG×1000

Materials and Methods①

- We measured the MPXI in 26 patients with AML treated at the Hyogo Cancer Center using an appropriately controlled ADVIA2120i.



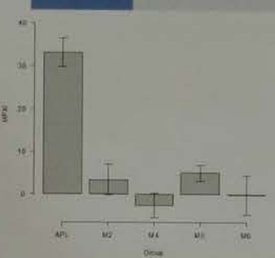
Materials and Methods②

- Statistical analyses were performed using the ANOVA and Tukey's tests. The significance level for each test was set at P values less than 0.01.
- All statistical analyses were performed with EZR , which is a graphical user interface for R.

Results①

The averages of the MPXI values in the different groups were as follows.

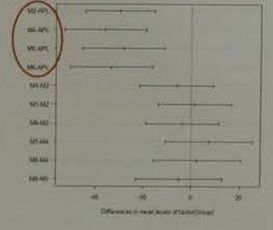
Group	The average of MPXI values	minimum	maximum	S.D.
APL (n=5)	33.12	24.1	44.8	7.46
AML-M2 (n=9)	3.48	-21.4	14.1	10.75
AML-M4 (n=4)	-2.55	-8.5	2.9	5.74
AML-M5 (n=4)	5.02	0.2	7.8	3.62
AML-M6 (n=4)	-0.13	-9.4	9.2	9.19



The averages of the MPXI values ± S.E.

- The P value of the one-way ANOVA was 0.00001.
- The difference in the average MPXI values between 5 groups were statistically significant .

Results②



The difference in the average MPXI values between the APL group and the other AML groups was statistically significant .

Discussion

- These data suggest that MPXI values are useful for the diagnosis of APL or the other AML subtypes. Interestingly, MPXI is effective in the differential diagnosis in the case that is difficult to diagnose morphologically including APL variant and the M2.
- It was thought that the remarkable high price of MPXI reflected abnormal granule (MPO strong positive granule) of APL.

Conclusion

- We measured the MPXI in patients with AML using ADVIA2120i.
- The MPXI in APL patients was higher in a statistical significance than that in patients with the other types of AML.
- MPXI values are useful for the diagnosis of AML subtypes.

matology

C-36

PC-36

The significance of medic
participating in confere

ose

there are few reports concerning hospitals regularly holding a nce on bone marrow (BM) examination and where medical ogists (MTs) participate. In our hospital, the conference has been held eek since 1985, and in addition to hematologists and pathologists, MTs ate there and give explanations on myelogram and morphological . In this instance, we'd like to report on the benefits and significance of rticipating in conferences on BM with representative case reports nced in 2015.

ect and methods

ere 197 cases discussed in the conference in 2015 out of 456 ed specimens. In these cases we'd like to show 2 cases that the nce was meaningful for diagnoses.

1

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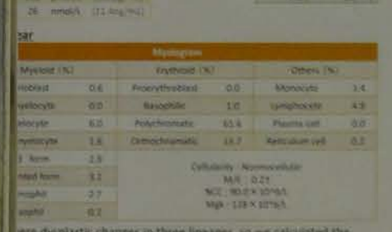
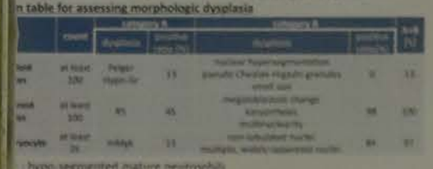
ear



ere dysplastic changes in three lineages, so we calculated the age of dysplastic cells of each lineage with our own table for assessing logic dysplasia according to the atlas on morphological diagnosis.

n table for assessing morphologic dysplasia

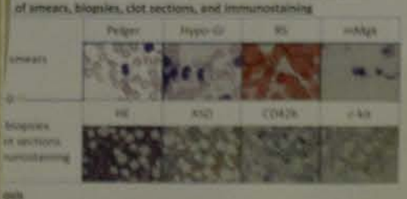


: hypo-segmented mature neutrophils
Gr : degranulation (+ or hypogranular neutrophils)
ng siderobla
micromegakaryocytes

tions and biopsies

poietic cells slightly increased. G/E is 1/2~3. Erythroid precursors ol, and had megaloblastoid change and RSs in iron staining. MgKs. Ly increased (63/mm²), and mMgk or non-tubulated MgK existed.

of smears, biopsies, clot sections, and immunostaining



osis

myelodysplastic syndrome refractory cytopenia with multilineage dysplasia (RCMD)

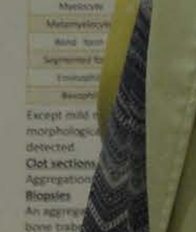
About the atla
In Japan we categor
atlas. This atlas says mo
into category A that is hig
that is less specific but ind
The atlas also says if morp
one linage more than 10%,
dysplasia.

Case 2

A 60s man, developed
transformation to Diffuse
exacerbation speed and
operated.

Blood test

BM smear



Except mild
morphologic
detected.

Clot sections

Aggregation

Biopsies

An aggreg
bone trab

Immunos

bcl-2 : pos

Images

Dis

BM

Co

Hematology

PC-35

INTERNATIONAL UNIVERSITY OF HEALTH AND WELFARE

FIR haploinsufficiency switches PKM1 to PKM2 in mice thymic lymphoma revealed by quantitative proteomic analysis

Asako KIMURA[1], Kouichi KITAMURA[2,3], Guzhalinue AILIKEN[2], Mamoru SATOH[4], Nobuko TANAKA[2], Fumio NOMURA[4], Kazuyuki MATSUSHITA[2,3]

1. Department of Medical Science Technology, Narita School of Health Sciences, International University of Health and Welfare.
2. Department of Molecular Diagnosis, Graduate School of Medicine, Chiba University,
3. Division of Laboratory Medicine, Chiba University Hospital,
4. Divisions of Clinical Mass Spectrometry and Clinical Genetics, Chiba University Hospital.

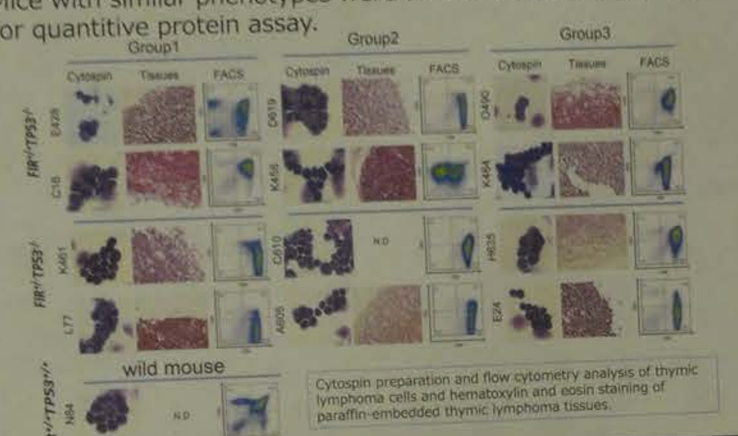
Introduction

- ✓ Pyruvate kinase (PK) M2 is activated in cancers through alternative splicing.
- ✓ An alternatively spliced form of FUSE-binding protein (FBP)-interacting repressor (FIR), a transcriptional repressor of the *c-myc* gene, is activated as a dominant negative in several cancers.
- ✓ $FIR^{+/-}TP53^{-/-}$ mice generated as previously described (Matsushita K et.al, Oncotarget 2015(6)) frequently developed thymic lymphoma and/or T cell type acute lymphoblastic lymphoma (T-ALL).
- ✓ To examine the mechanism of FIR's role in tumor development, the quantitative protein profile was revealed by six-plex tandem mass tags (TMT) in the $FIR^{+/-}TP53^{-/-}$ mice thymic lymphoma model.

Material

Mice Thymus

- ✓ Twenteen thymic lymphoma tissues (six $FIR^{+/+}TP53^{-/-}$ and six $FIR^{+/-}TP53^{-/-}$)
- ✓ Normal thymus from $FIR^{+/+}TP53^{+/+}$ as internal controls
- ✓ Mice with similar phenotypes were divided into three groups for quantitive protein assay.

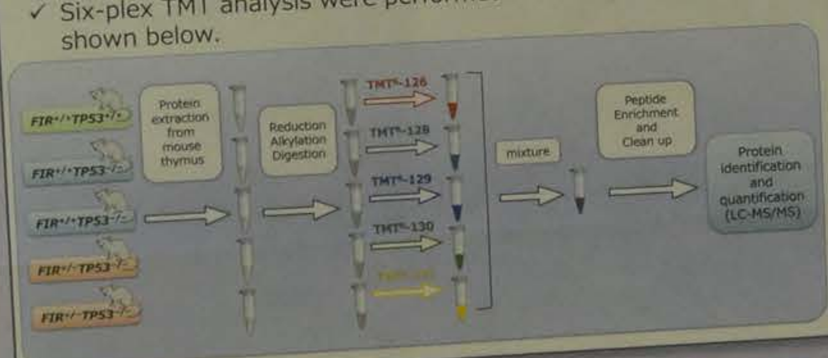


Cytospin preparation and flow cytometry analysis of thymic lymphoma cells and hematoxylin and eosin staining of paraffin-embedded thymic lymphoma tissues.

Methods

Quantitive Protein Assay

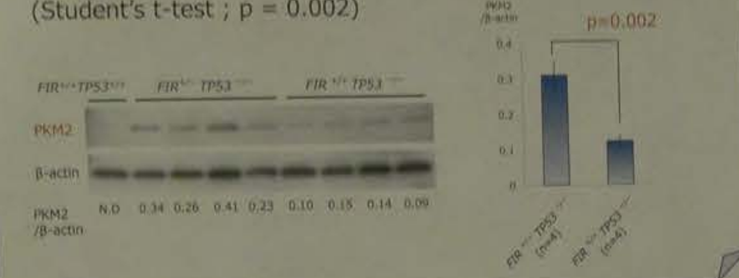
- ✓ Six-plex TMT analysis were performed according to the protocol shown below.



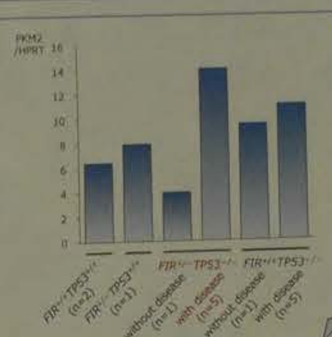
Results

- ✓ TMT analysis revealed **45 proteins were quantified 1.5 times higher/ lower in $FIR^{+/-}TP53^{-/-}$ samples** compared with normal $FIR^{+/+}TP53^{+/+}$.
- ✓ PKM2 was activated **at protein and mRNA levels** in thymic lymphoma of $FIR^{+/-}TP53^{-/-}$ mice .

Western blotting confirmed that **PKM2 was overexpressed 2.5 fold in the thymic lymphoma of $FIR^{+/-}TP53^{-/-}$ mice** compared with $FIR^{+/+}TP53^{-/-}$ mice. (Student's t-test ; p = 0.002)

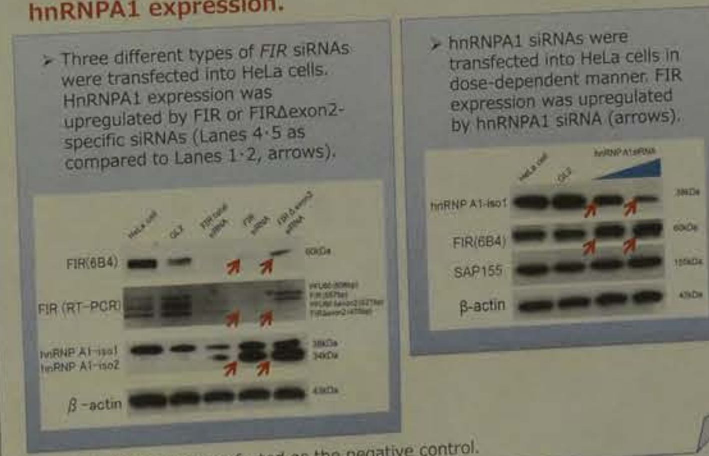


qRT-PCR confirmed that *PKM2* mRNA expression was **significantly upregulated in thymic lymphoma of $FIR^{+/-}TP53^{-/-}$ mice** sorted by flow cytometry.



Knockdown of FIR by siRNA suppressed hnRNPA1 expression in HeLa cells, indicating that **altered FIR expression affected PKM1/PKM2 splicing through hnRNPA1 expression.**

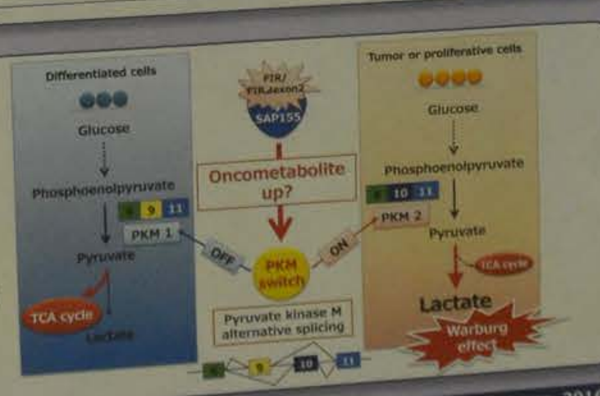
- ➤ Three different types of *FIR* siRNAs were transfected into HeLa cells. HnRNPA1 expression was upregulated by FIR or FIRΔexon2-specific siRNAs (Lanes 4-5 as compared to Lanes 1-2, arrows).
- ➤ hnRNPA1 siRNAs were transfected into HeLa cells in dose-dependent manner. FIR expression was upregulated by hnRNPA1 siRNA (arrows).



The *GL2* siRNA was transfected as the negative control.

Conclusions

- ✓ ***FIR* haploinsufficiency promotes alternative splicing of PKM1 to PKM2** in mice thymic lymphoma cells prior to T-ALL cells potentially via at least partly **affecting hnRNPA1 expression.** (Shown at the right)
- ✓ Alternative splicing switch is required but **not sufficient for T-ALL progression in our mice model.**
- ✓ Our findings suggest that *FIR* and its related spliceosomes are potential therapeutic targets for cancers, including T-ALL.



The 32nd World Congress of Biomedical Laboratory Science, 2016

Hematology

PC-36

The significance of medical technologists (MTs) participating in conferences on bone marrow (BM)

Nozomi Aoyama

NTT Medical Center Tokyo

PC-36

Purpose

In Japan there are few reports concerning hospitals regularly holding a conference on bone marrow (BM) examination and where medical technologists (MTs) participate. In our hospital, the conference has been held every week since 1985, and in addition to hematologists and pathologists, MTs participate there and give explanations on myelogram and morphological findings. In this instance, we'd like to report on the benefits and significance of MTs participating in conferences on BM with representative case reports experienced in 2015.

Subject and methods

There were 197 cases discussed in the conference in 2015 out of 456 submitted specimens. In these cases we'd like to show 2 cases that the conference was meaningful for diagnoses.

Case 1

A 70s man was suspected for early gastric cancer by the result of gastrointestinal fiberscopy, so consulted to a gastroenterologist. In a preoperative examination he was found to have pancytopenia, so consulted to a hematologist and a BM examination was operated.

Blood test

CBC			Differentiation		
WBC	2.6	$10^9/L$	Myeloblast	0.0	%
RBC	2.31	$10^{12}/L$	Promyelocyte	0.0	%
HGB	6.6	g/dL	Myelocyte	0.5	%
HCT	20.9	%	Metamyelocyte	1.5	%
MCV	90.5	fL	Band form	1.0	%
MCH	28.6	pg	Segmented form	57.0	%
MCHC	31.6	g/dL	Lymphocyte	34.5	%
Plt	107	$10^9/L$	Monocyte	3.5	%
			Eosinophil	[illegible]	%
			Basophil	0.0	%
			NRBC	1.0	%

BM smear

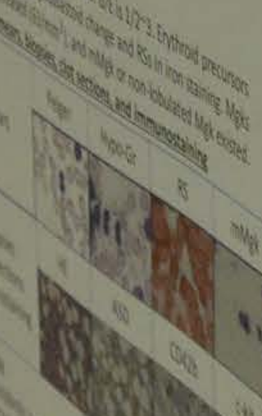
Myelogram

Myeloid (%)		Erythroid (%)		Others (%)	
Myeloblast	0.5	Proerythroblast	0.0	Monocyte	1.4
Promyelocyte	[illegible]	Basophilic	1.0	Lymphocyte	4.8
Myelocyte	[illegible]	Polychromatic	61.6	Plasma cell	0.0
Metamyelocyte	1.6	Orthochromatic	13.7	Reticulum cell	0.2
Band form	[illegible]				
Segmented form	[illegible]				
Eosinophil	[illegible]				
Basophil	[illegible]				

Cellularity : Normocellular
M/E : 0.23

There were dysplastic changes in three lineages, so we calculated the percentage of dysplastic cells of each lineage with our own table for assessing morphologic dysplasia according to the atlas on morphological diagnosis.

Our own table for assessing morphologic dysplasia



Diagnosis

Refractory cytopenia with multilineage dysplasia

About the atlas on morphological diagnosis

In Japan we categorize morphological dysplasia with this atlas. This atlas says morphological dysplasia can be devided into category A that is highly specific to MDS and category B that is less specific but indicates MDS if frequently detected. The atlas also says if morphological dysplasia is detected in one linage more than 10%, we can say the linage has a dysplasia.

(atlas on morphological diagnosis)

Case 2

A 60s man, developed Follicular lymphoma (FL) in 2002, was suspected for transformation to Diffuse Large B-cell Lymphoma(DLBCL) because of the exacerbation speed and his clinical condition. Thus a BM examination was operated.

Blood test

Biochemistry				CBC			Differentiation		
TP	56	g/L	(5.6g/dL)	WBC	3.9	$10^9/L$	Myeloblast	0.0	%
Alb	40	g/L	(4.0g/dL)	RBC	2.79	$10^{12}/L$	Promyelocyte	0.0	%
UA	360	μmol/L	(6.0mg/dL)	Hgb	9.2	g/dL	Myelocyte	0.5	%
UN	7.5	mmol/L	(21.1mg/dL)	Hct	28.8	%	Metamyelocyte	0.0	%
Cre	90	μmol/L	(1.0mg/dL)	MCV	103.2	fL	Band form	5.0	%
ALP	390	U/L	(390U/L)	MCH	33.0	pg	Segmented form	74.5	%
γ-GT	14	U/L	(14U/L)	MCHC	31.9	g/dL	Lymphocyte	13.0	%
LDH	283	U/L	(283U/L)	Plt	98	$10^9/L$	Monocyte	7.0	%
sIL-2R	1573	U/mL	(1573U/mL)				Eosinophil	0.0	%
							Basophil	0.0	%

BM smear

Myelogram

Myeloid (%)		Erythroid (%)		Others (%)	
Myeloblast	1.2	Proerythroblast	0.0	Monocyte	4.1
Promyelocyte	0.3	Basophilic	0.3	Lymphocyte	12.3
Myelocyte	15.3	Polychromatic	29.9	Plasma cell	0.4
Metamyelocyte	8.1	Orthochromatic	0.8	Reticulum cell	0.1
Band form	10.6				
Segmented form	13.9				
Eosinophil	2.4				
Basophil	0.0				

Cellularity : Hypocellular
M/E : 1.68
NCC : $35.0 \times 10^9/L$
Mgk : (-)

Except mild megaloblastoid change of erythroid precursors, marked morphological dysplasia for two other lineages and atypical cells were not detected.

Clot sections

Aggregations of small lymphocyte-like cells were partially detected.

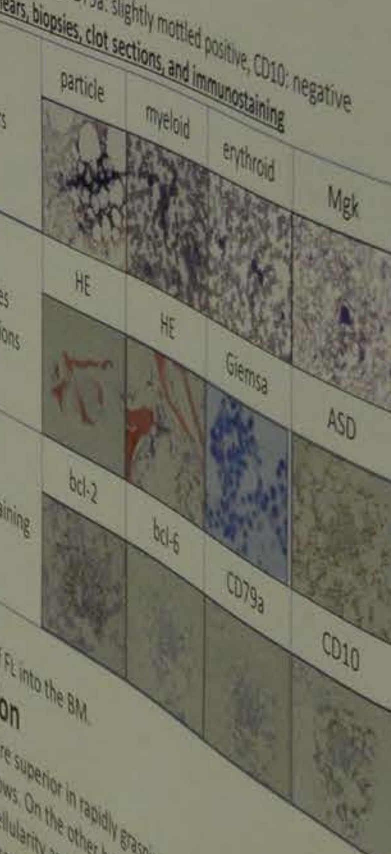
Biopsies

An aggregation of small lymphocyte-like cells were detected in an edge of bone trabecula.

Immunostaining

bcl-2: positive, bcl-6 and CD79a: slightly mottled positive, CD10: negative

Images of smears, biopsies, clot sections, and immunostaining



Diagnosis

Infiltration of FL into the BM.

Discussion

BM smears are superior in rapidly grasping morphological findings of each cell as Case 1 shows. On the other hand, clot sections and biopsies are superior in evaluating cellularity and proving lymphoma cell infiltration as Case 2 shows. Through these cases we could clearly recognize the merits of each method of BM examination.

Conclusion

It is very meaningful to grasp whether myelogram and morphological findings correspond to histological ones through the conference. Moreover, the conference leads MTs to keep motivated and to improve the clinicopathological knowledge for understanding the histological findings and process of the diagnosis. Thus, we'd like to recommend our activities to other institutions.

Hematology

PC-36

PC-36

The significance of medical technologists (MTs) participating in conferences on bone marrow (BM)

Nozomi Aoyama

NTT Medical Center Tokyo

Purpose

In Japan there are few reports concerning hospitals regularly holding a conference on bone marrow (BM) examination and where medical technologists (MTs) participate. In our hospital, the conference has been held every week since 1985, and in addition to hematologists and pathologists, MTs participate there and give explanations on myelogram and morphological findings. In this instance, we'd like to report on the benefits and significance of MTs participating in conferences on BM with representative case reports experienced in 2015.

Subject and methods

There were 197 cases discussed in the conference in 2015 out of 456 submitted specimens. In these cases we'd like to show 2 cases that the conference was meaningful for diagnoses.

Case 1

A 70s man was suspected for early gastric cancer by the result of gastrointestinal fiberscopy, so consulted to a gastroenterologist. In a preoperative examination he was found to have pancytopenia, so consulted to a hematologist and a BM examination was operated.

Blood test

Biochemistry		SI	(non SI)
TP	63	g/L	(6.3g/dL)
Alb	43	g/L	(4.3g/dL)
UA	380	μmol/L	(6.3mg/dL)
UN	5.8	mmol/L	(16.3mg/dL)
Cre	70	μmol/L	(0.74mg/dL)
LDH	255	IU/L	(255IU/L)
Fe	45	μmol/L	(252μg/dL)
UIBC	4	μmol/L	(22μg/dL)
Fer	461	μg/L	(461ng/dL)
VB12	280	pmol/L	(380pg/mL)
folic acid	26	nmol/L	(11.4ng/mL)

CBC		
WBC	2.6	10^9/L
RBC	2.31	10^{12}/L
HGB	6.6	g/dL
HCT	20.9	%
MCV	90.5	fL
MCH	28.6	pg
MCHC	31.6	g/dL
Plt	107	10^9/L

Differentiation		
Myeloblast	0.0	%
Promyelocyte	0.0	%
Myelocyte	0.5	%
Metamyelocyte	1.5	%
Band form	1.0	%
Segmented form	57.0	%
Lymphocyte	34.5	%
Monocyte	3.5	%
Eosinophil	16.0	%
Basophil	0.0	%
NRBC	1.0	%

BM smear

Myelogram					
Myeloid (%)		Erythroid (%)		Others (%)	
Myeloblast	0.6	Proerythroblast	0.0	Monocyte	1.4
Promyelocyte	0.0	Basophilic	1.0	Lymphocyte	4.8
Myelocyte	6.0	Polychromatic	61.6	Plasma cell	0.0
Metamyelocyte	1.6	Orthochromatic	13.7	Reticulum cell	0.2
Band form	2.9	Cellularity : Normocellular M/E : 0.23 NCC : 90.0 × 10^9/L Mgk : 128 × 10^6/L			
Segmented form	3.2				
Eosinophil	2.7				
Basophil	0.2				

There were dysplastic changes in three lineages, so we calculated the percentage of dysplastic cells of each lineage with our own table for assessing morphologic dysplasia according to the atlas on morphological diagnosis.

Our own table for assessing morphologic dysplasia

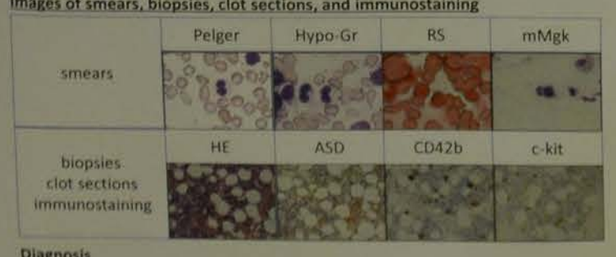
	count	category A		category B		A+B [%]
		dysplasia	positive ratio [%]	dysplasia	positive ratio[%]	
Myeloid series	at least 100	Pelger Hypo-Gr	13	nuclear hypersegmentation pseudo Chediak-Higashi granules small size	0	13
Erythroid series	at least 100	RS	45	megaloblastoid change karyorrhexis multinuclearity	98	100
Megakaryocyte	at least 25	mMgk	13	non-lobulated nuclei multiple, widely-separated nuclei	84	97

※Pelger : hypo-segmented mature neutrophils
※Hypo-Gr : degranulation (a- or hypogranular neutrophils)
※RS : ring sideroblasts
※mMgk : micromegakaryocytes

Clot sections and biopsies

Hematopoietic cells slightly increased. G/E is 1/2~3. Erythroid precursors increased, and had megaloblastoid change and RSs in iron staining. Mgks obviously increased (63/mm^2), and mMgk or non-lobulated Mgk existed.

Images of smears, biopsies, clot sections, and immunostaining



Diagnosis

myelodysplastic syndrome refractory cytopenia with multilineage dysplasia; MDS RCMD.

About the atlas on morphological diagnosis

In Japan we categorize morphological dysplasia with this atlas. This atlas says morphological dysplasia can be devided into category A that is highly specific to MDS and category B that is less specific but indicates MDS if frequently detected. The atlas also says if morphological dysplasia is detected in one linage more than 10%, we can say the linage has a dysplasia.

(atlas on morphological diagnosis)

Case 2

A 60s man, developed Follicular Lymphoma (FL) in 2002, was suspected for transformation to Diffuse Large B-cell Lymphoma(DLBCL) because of the exacerbation speed and his clinical condition. Thus a BM examination was operated.

Blood test

Biochemistry		SI	(non SI)
TP	56	g/L	(5.6g/dL)
Alb	40	g/L	(4.0g/dL)
UA	360	μmol/L	(6.0mg/dL)
UN	7.5	mmol/L	(21.1mg/dL)
Cre	90	μmol/L	(0.97mg/dL)
ALP	390	IU/L	(390IU/L)
γ-GT	14	IU/L	(14IU/L)
LDH	283	IU/L	(283IU/L)
sIL-2R	1572	U/mL	(1572U/mL)

CBC		
WBC	3.9	10^9/L
RBC	2.79	10^{12}/L
Hgb	9.2	g/dL
Hct	28.8	%
MCV	103.2	fL
MCH	33.0	pg
MCHC	31.9	g/dL
Plt	98	10^9/L

Differentiation		
Myeloblast	0.0	%
Promyelocyte	0.0	%
Myelocyte	0.5	%
Metamyelocyte	0.0	%
Band form	5.0	%
Segmented form	74.5	%
Lymphocyte	13.0	%
Monocyte	7.0	%
Eosinophil	0.0	%
Basophil	0.0	%

BM smear

Myelogram					
Myeloid (%)		Erythroid (%)		Others (%)	
Myeloblast	1.2	Proerythroblast	0.0	Monocyte	4.1
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Metamyelocyte	8.1	Orthochromatic	0.8	Reticulum cell	0.1
Band form	10.6	Cellularity : Hypocellular M/E : 1.68 NCC : 35.0 × 10^9/L Mgk : (+)			
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Except mild megaloblastoid change of erythroid precursors, marked morphological dysplasia for two other lineages and atypical cells were not detected.

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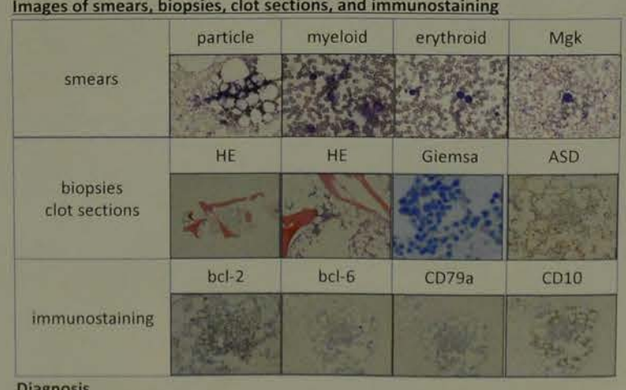

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Conclusion

It is very meaningful to grasp whether myelogram and morphological findings correspond to histological ones through the conference. Moreover, the conference leads MTs to keep motivated and to improve the clinicopathological knowledge for understanding the histological findings and process of the diagnosis. Thus, we'd like to recommend our activities to other institutions.

Efficacy of CellaVision® Competency Software
educational tool for learning blood cell morpho

Asami Naito[1,4], Etsu Suzuki[2], Michikuni Ishijima[1], Takayuki Yamamoto[1], Ryou Sunaga[1], Kazumasa Isobe[4]

1 : Tsukuba i-Laboratory LLP, Japan (e-mail: naito@tsukuba
2 : Tsukuba Medical Laboratory of Education and Rese
3 : LSI Medience Corpora
4 : Department of Laboratory Medicine, University of Tsuk

d cross training are required for developing
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CS) is a program for education and
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ve evaluated the efficacy of this program.

was to assess the effect of an educational
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s study participants.

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med the skill test once a week for 4 weeks.

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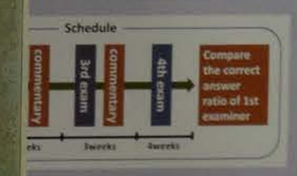
ipants were shown their results by the
l-by-cell comparison and their achievement
articipants.

d by a grant from Tsukuba Medical
and Research.

Schedule



ean correct answer ratio improved from
f the 4th week participants reached to

Results2

As shown in Fig 2, first week classification performance of th
participants was variable, but by the 4th week they had becor
more consistently correct.

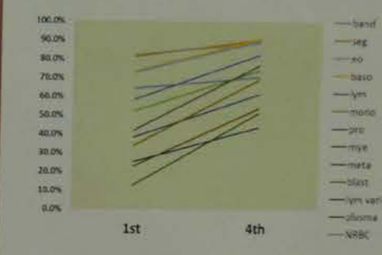


Fig 2 Changes in the correct answer ratio for the cell categor

Fig 3 shows the changes in cell classification ability by cell samp
Cell classification ability varied during the first week, but by the
week the cell classification scores became less variable.

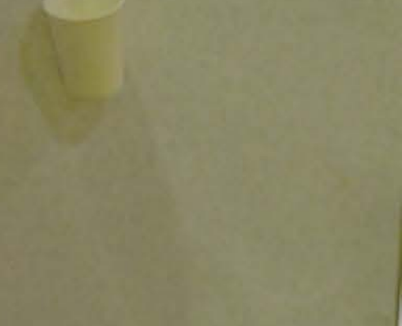
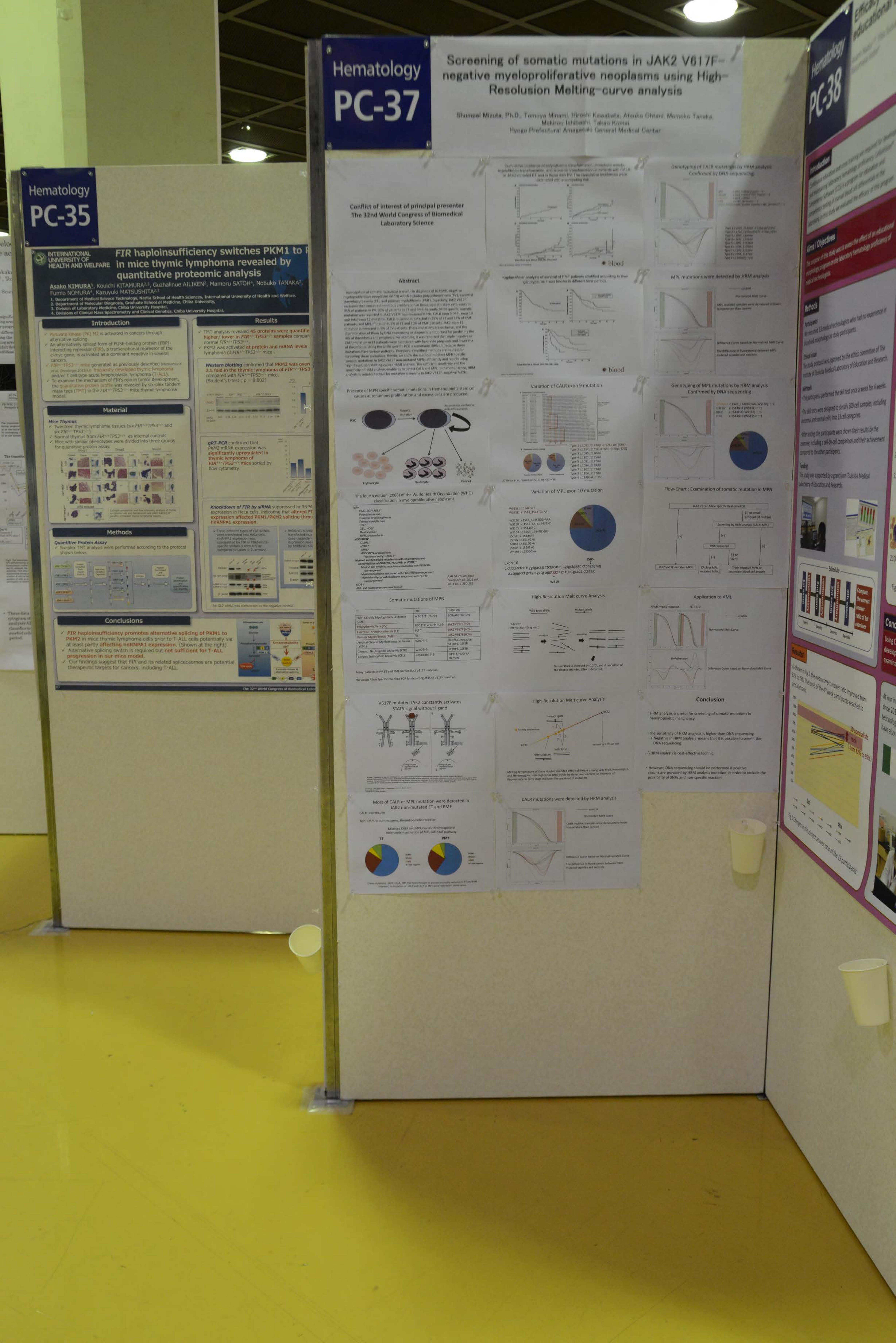


Fig 3 Changes in cell classification of the cell samples (Promyelo

Conclusion

Using CCS helped inexperienced clinical laboratory technicians to develop high levels of proficiency in their blood cell morphological examination accuracy.

At our institute we have held a foreign trainee invitation program since 2011 and have used this software to train medical technologists from Nigeria, Cameroon, and the Philippines. They have also appreciated the game-like, active, self-learning style of CC


Hematology
PC-35
INTERNATIONAL UNIVERSITY OF HEALTH AND WELFARE
FIR haploinsufficiency switches PKM1 to P
in mice thymic lymphoma revealed by
quantitative proteomic analysis
Asako KIMURA1, Kouichi KITAMURA2,3, Guzhalinue AILIKEN2, Mamoru SATOH4, Nobuko TANAKA2, Fumio NOMURA4, Kazuyuki MATSUSHITA2,3
1. Department of Medical Science Technology, Narita School of Health Sciences, International University of Health and Welfare.
2. Department of Molecular Diagnosis, Graduate School of Medicine, Chiba University.
3. Division of Laboratory Medicine, Chiba University Hospital.
4. Divisions of Clinical Mass Spectrometry and Clinical Genetics, Chiba University Hospital.
Introduction
Results
Material
Mice Thymus
Methods
Quantitive Protein Assay
Conclusions
FIR haploinsufficiency promotes alternative splicing of PKM1 to PKM2 in mice thymic lymphoma cells prior to T-ALL cells potentially via at least partly affecting hnRNPA1 expression. (Shown at the right)
Alternative splicing switch is required but not sufficient for T-ALL progression in our mice model.
Our findings suggest that FIR and its related spliceosomes are potential therapeutic targets for cancers, including T-ALL.
Hematology
PC-37
Screening of somatic mutations in JAK2 V617F-negative myeloproliferative neoplasms using High-Resolution Melting-curve analysis
Shumpei Mizuta, Ph.D., Tomoya Minami, Hiroshi Kawabata, Atsuko Ohtani, Momoko Tanaka, Makirou Ishibashi, Takao Komai
Hyogo Prefectural Amagasaki General Medical Center
Conflict of interest of principal presenter
The 32nd World Congress of Biomedical Laboratory Science
Abstract
Presence of MPN specific somatic mutations in Hematopoietic stem cell causes autonomous proliferation and excess cells are produced.
The fourth edition (2008) of the World Health Organization (WHO) classification in myeloproliferative neoplasms
Somatic mutations of MPN
V617F mutated JAK2 constantly activates STAT5 signal without ligand
Most of CALR or MPL mutation were detected in JAK2 non-mutated ET and PMF
Genotyping of CALR mutations by HRM analysis Confirmed by DNA sequencing
MPL mutations were detected by HRM analysis
Variation of CALR exon 9 mutation
Genotyping of MPL mutations by HRM analysis Confirmed by DNA sequencing
Variation of MPL exon 10 mutation
Flow-Chart : Examination of somatic mutation in MPN
High-Resolution Melt curve Analysis
Application to AML
Conclusion
・HRM analysis is useful for screening of somatic mutations in hematopoietic malignancy.
・The sensitivity of HRM analysis is higher than DNA sequencing.
→ Negative in HRM analysis means that it is possible to ommit the DNA sequencing.
∴HRM analysis is cost-effective technic.
・However, DNA sequencing should be performed if positive results are provided by HRM analysis mutation; in order to exclude the possibility of SNPs and non-specific reaction.
CALR mutations were detected by HRM analysis
Hematology
PC-38
Introduction
Aims / Objectives
Methods
Results1
Fig.1 Changes in the correct answer ratio of the 13 participants

Hematology

PC-38

Efficacy of CellaVision® Competency Software as an educational tool for learning blood cell morphology

Asami Naito[1,4], Etsu Suzuki[2], Michikuni Ishijima[1], Takayuki Yamamoto[1], Ryou Sunaga[3], Kazumasa Isobe[4]

1 : Tsukuba i-Laboratory LLP, Japan (**e-mail: naito@tsukuba-i-lab.com**)
2 : Tsukuba Medical Laboratory of Education and Research, Japan
3 : LSI Medience Corporation, Japan
4 : Department of Laboratory Medicine, University of Tsukuba, Japan

Introduction

Continuous education and cross training are required for developing and maintaining laboratory hematology proficiency. CellaVision® Competency Software (CCS) is a program for education and competency testing of manual blood cell differentials in the laboratory. In this study we evaluated the efficacy of this program.

Aims / Objectives

The purpose of this study was to assess the effect of an educational morphology program on the laboratory hematology proficiency of medical technologists.

Methods

Participants
We recruited 13 medical technologists who had no experience in blood cell morphology as study participants.

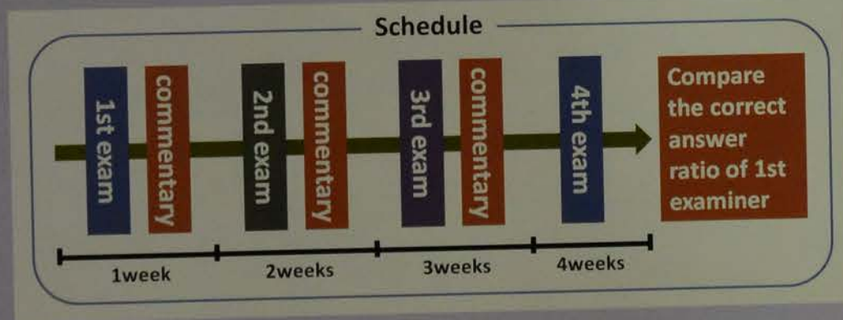
Ethical issue
The study protocol was approved by the ethics committee of The Institute of Tsukuba Medical Laboratory of Education and Research.

Methods
・The participants performed the skill test once a week for 4 weeks.

・The skill tests were designed to classify 300 cell samples, including abnormal and normal cells, into 13 cell categories.

・After testing, the participants were shown their results by the examiner, including a cell-by-cell comparison and their achievement compared to the other participants.

Funding
This study was supported by a grant from Tsukuba Medical Laboratory of Education and Research.



Results1

As shown in Fig 1, the mean correct answer ratio improved from 62% to 78%. The levels of the 4th week participants reached to specialist rank.

Fig 1 Changes in the correct answer ratio of the 13 participants

Results2

As shown in Fig 2, first week classification performance of the participants was variable, but by the 4th week they had become more consistently correct.

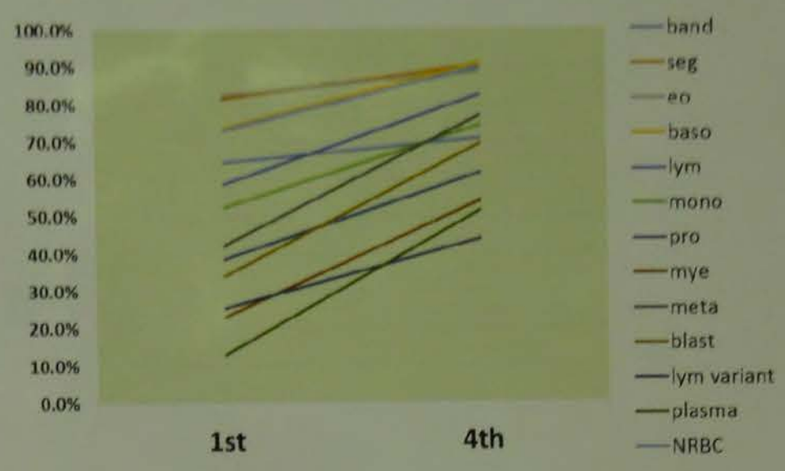
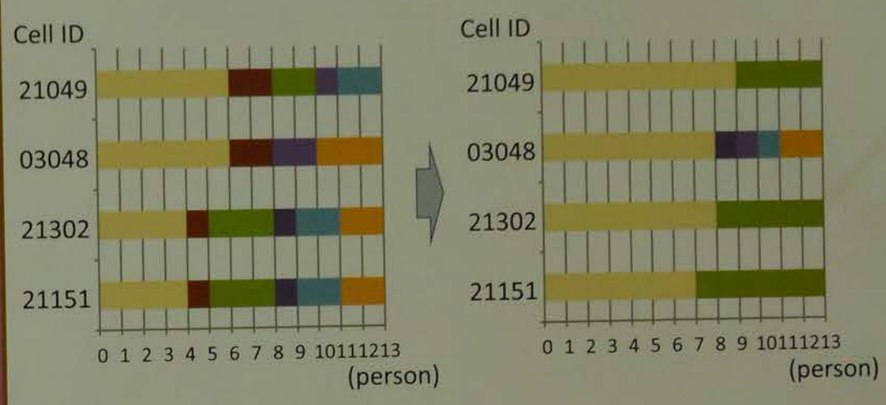


Fig 2 Changes in the correct answer ratio for the cell categories

Fig 3 shows the changes in cell classification ability by cell samples. Cell classification ability varied during the first week, but by the 4th week the cell classification scores became less variable.



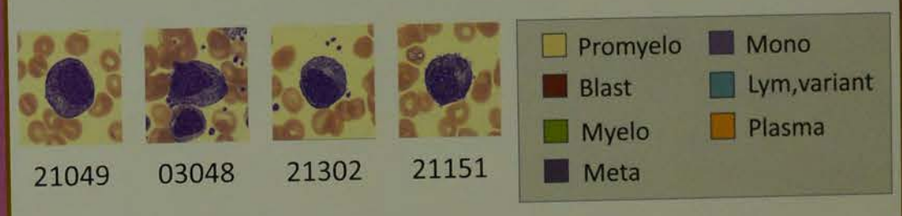


Fig 3 Changes in cell classification of the cell samples (Promyelo)

Conclusion

Using CCS helped inexperienced clinical laboratory technicians to develop high levels of proficiency in their blood cell morphological examination accuracy.

At our institute we have held a foreign trainee invitation program since 2011 and have used this software to train medical technologists from Nigeria, Cameroon, and the Philippines. They have also appreciated the game-like, active, self-learning style of CCS.

Hematology

PC-39

Assessment of cilostazol inhibition using whole blood platelet function tests: a translational study

Kaneo Satoh[*1], Masato Ohta[*1], Isao Fukasawa[*2], Kazuya Hosokawa[*3], Tomoko Oonishi[*3], Yukio Ozaki[*4]

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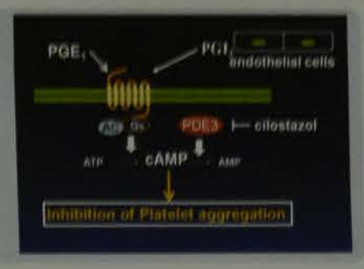
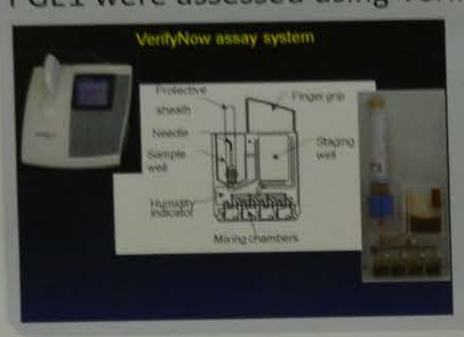
[*2] Division of Neurosurgery, Kofu Jounan Hospital, 53-1 Uemachi, Kofu, Yamanashi, Japan.
[*3] Research Institute, Fujimori Kogyo Co., 1-10-1 Sachiura, Yokohama, Kanagawa, Japan.
[*4] Fuefuki Chuo Hospital, 47-1 Yokkaichiba, Isawa, Fuefuki, Yamanashi, Japan.

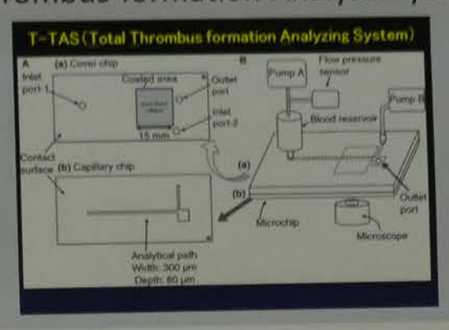
UNIVERSITY OF YAMANASHI

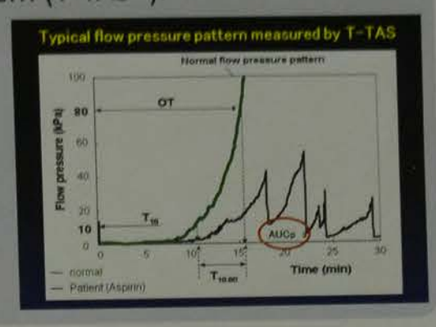
Introduction: Cilostazol inhibits phosphodiesterase, increases intracellular cyclic AMP and inhibits platelet aggregation, particularly in the presence of prostaglandin E_1 (PGE_1). We previously reported that the addition of low concentrations of PGE_1, which did not affect platelet aggregation per se, potentiated the inhibitory effects of cilostazol on platelet-rich plasma based- platelet aggregation (Thromb Res. 2012; 130: 616). This study aimed to establish a cilostazol monitoring assay based on whole blood samples.



Methods: Whole blood samples were taken from the antecubital veins with vacutainer tubes containing 3.13% trisodium citrate (9:1, v/v) (Nipro, Osaka, Japan) and hirudin (20 μg/ml final concentration, Verum Diagnostica GmbH, Munich, Germany). The tubes were then stored at room temperature for 1 h without agitation. Whole blood samples without or with several PGE1 were assessed using VerifyNow®, Multiplate® and Total Thrombus-formation Analysis System (T-TAS®).







Results: Cilostazol was no inhibition without PGE_1 measured by the three tests in *in vitro* and *ex vivo* studies. *In vitro* platelet aggregation, in the presence of cilostazol and PGE_1, measured by the aspirin, IIb/IIIa and P2Y12 tests, decreased by 19%, 44% and 9%, respectively (Figure 1, A-C). *Ex vivo* platelet aggregation, measured by these tests, decreased by 8%, 46% and 23%, respectively (Figure 2). However, platelet aggregation inhibition was not assessed by multiplate, thrombus formation or T-TAS both *in vitro* (Figure 1, D-H) and *ex vivo* (data not shown). Compared with pre-dosing blood samples, blood samples from cerebral infarction patients after dosing showed significant platelet aggregation inhibition in the presence of 3 nM (36%) and 10 nM PGE_1 (75%) (Figure 3).

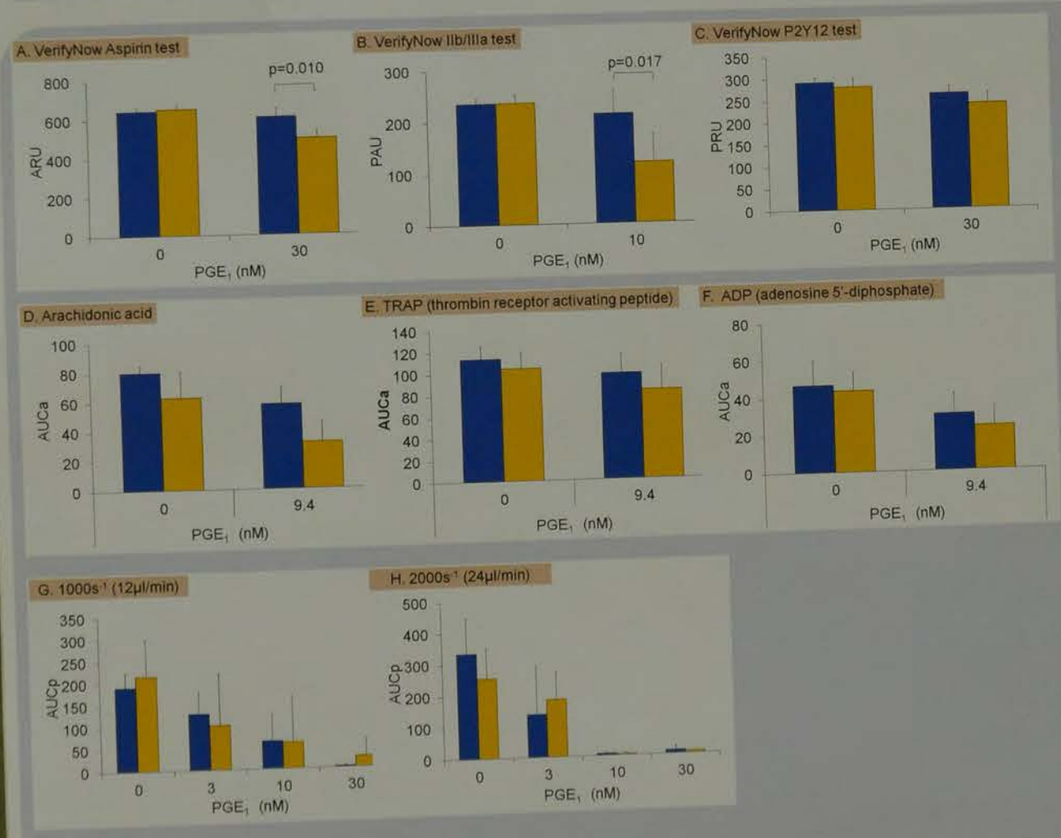


Figure 1. Assessment of *in vitro* effects of cilostazol using VerifyNow, Multiplate and T-TAS in healthy volunteers.
Citrate-treated whole blood was incubated without (blue) or with (3 μM, orange) cilostazol for 2 min at 37° C and then incubated without or with indicated concentrations of PGE_1 for 2 min at 37° C. The vacutainer tube was inserted into the VerifyNow Aspirin (A), IIb/IIIa (B) and P2Y12 tests (C). Hirudin-treated whole blood was equally diluted with 300 μL saline preheated to 37° C in the test cell of Multiplate for 3 min. The diluted blood was incubated without (blue) or with (3 μM, orange) cilostazol for 2 min at 37° C and then incubated without or with 9.4 nM PGE_1 (ADPtestHS) for 2 min at 37° C. Platelet aggregation assessed by increasing impedance was induced by 0.5 mM arachidonic acid (D), 32.2 μM TRAP (E) and 6.5 μM ADP (F). Hirudin-treated whole blood was incubated without (blue) or with (3 μM, orange) cilostazol for 2 min at 37° C, then incubated without or with indicated concentrations of PGE_1 for 2 min at 37° C. The blood was perfused into a PL-chip at flow rates of 12 μL min^{-1} (G) and 24 μL min^{-1} (H). The thrombus formation was quantitatively assessed using flow pressure, and all results are expressed as mean ± SD (n = 4–5).

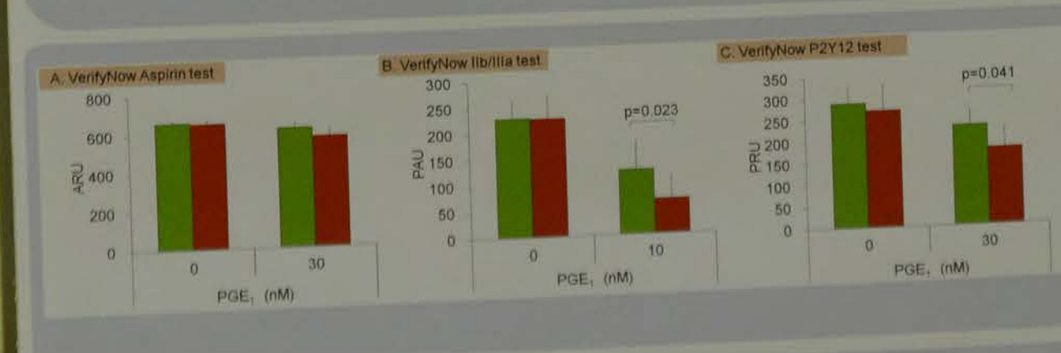


Figure 2. Assessment of the *ex vivo* effects of cilostazol in healthy volunteers using VerifyNow.
Citrate-treated whole blood was withdrawn before (green) and 2 h after (red) administration of 100 mg of cilostazol. The blood was incubated without or with the indicated concentrations of PGE1 for 2 min at 37° C, and the vacutainer tube was inserted into the VerifyNow Aspirin (A), IIb/IIIa (B) and P2Y12 tests (C). All results are expressed as mean ± SD (n = 4–9).

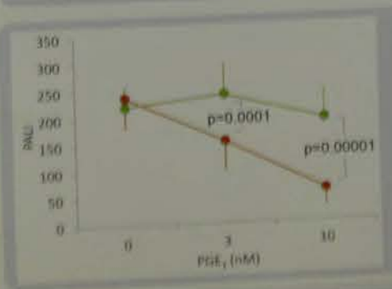


Figure 3. Assessment of the *ex vivo* effects of cilostazol in patients with cerebral infarction using on VerifyNow.
Citrate-treated whole blood was withdrawn before (green) and 2 h after (red) the administration of 50 mg of cilostazol. Blood was incubated without or with 10 nM PGE_1 for 2 min at 37° C, and the vacutainer tube was inserted into the VerifyNow IIb/IIIa test (n = 26). All results are expressed as mean ± SD.

Conclusion: Platelet aggregation measured by VerifyNow using the IIb/IIIa test in the presence of 10 nM PGE_1 is the most suitable tool for assessing the inhibitory effects of cilostazol.

A-3 A-3

PC-40

Performance of prothrombin time for monitoring of patients treated with rivaroxaban

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Abstract

Background : Prothrombin time (PT) can provide a qualitative assessment of the relative intensity of anticoagulation by rivaroxaban. More than ten types of assay are available for the measurement of PT in clinical settings, but it is not yet fully understood whether their interactions with rivaroxaban are uniform or inconsistent. Furthermore, the lot-to-lot variation of the assays has not been reported.

Method : We examined 139 blood samples from patients taking rivaroxaban. We measured PT using five different commercially available assays. We also evaluated the estimated rivaroxaban concentration using a chromogenic anti-factor Xa assay. Moreover, we examined whether there was lot-to-lot variation for each of the five assays, using two lots of each assay to measure PT in 34 samples.

Result : The median estimated concentration of rivaroxaban was 192 ng/mL (interquartile range 85 – 284 ng/mL). The correlation coefficient (r) between PT and the estimated concentrations of plasma rivaroxaban was follows : Thromborel S, $r = 0.768$; Thrombocheck PT, $r = 0.861$; Coagpia PT-N, $r = 0.909$; Neoplastin Plus, $r = 0.802$; and Triniclot PT Excel S, $r = 0.870$. The gradients of the regression plots differed more than four-fold, and the standard deviation of the regression line ranged from 1.001 to 2.980, which tended to be higher for the assays with the higher regression slope gradients. The existence of lot-to-lot variation measured using two different lots of each PT assay was observed with most assays.

Conclusion : The estimated concentration of plasma rivaroxaban varied greatly depending on the PT assay, so the PT measured in patients treated with rivaroxaban should be interpreted with caution.

Object

We clarify the performance of PT for monitoring of patients treated with rivaroxaban.

Methods

Collection

139 blood samples from patients taking rivaroxaban

Measured

PT : Thromborel S® (Sysmex)
Tbrombocheck PT® (Sysmex)
Coagpia PT-N® (Sekisui Medical)
Neoplastin Plus® (Diagnostica Stago)
Triniclot PT Excel S® (Trinity Biotech)

Estimated concentration of plasma rivaroxaban :
STA-Liquid Anti-Xa kit® (Diagnostica Stago)

All assays were conducted using a Coapresta 2000® (Sekisui Medical)

Lot-to-lot variation

two lots of each assay and PT in 34 blood samples

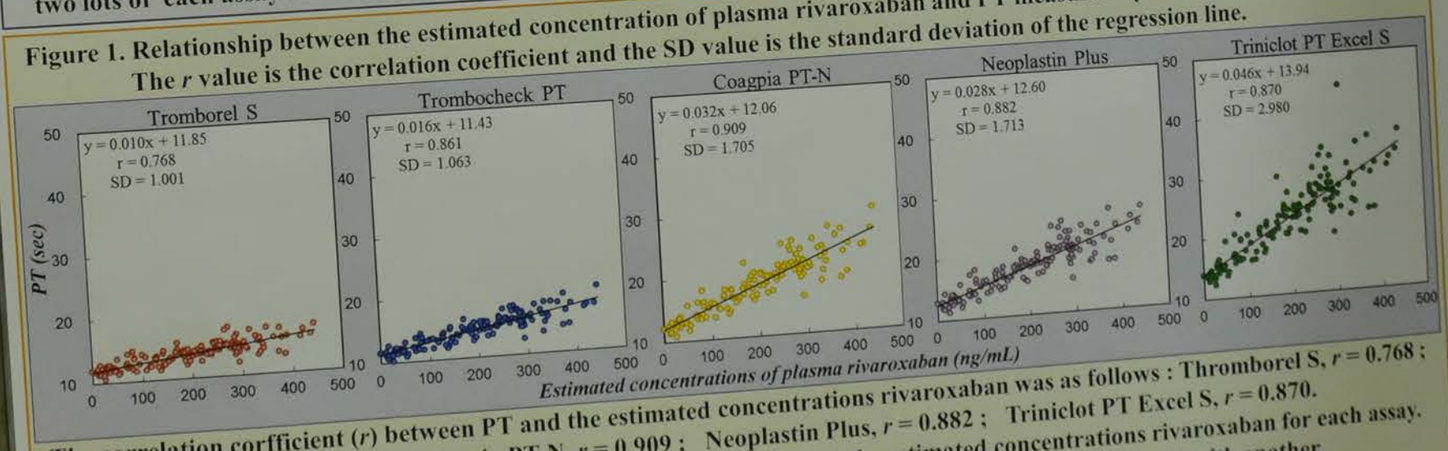
Results

Table 1. Baseline Characteristics of Blood Samples

		Dosage of rivaroxaban		
		10mg OD	15 mg OD	
	n = 139	n = 44 (32%)	n = 95 (68%)	p value
Age (y)	66.5 ± 8.5	69.0 ± 9.5	65.4 ± 7.7	0.011
Males n (%)	108 (78%)	33 (75%)	75 (79%)	0.600
eGFR (mL/min/1.73m^2)	68.5 ± 17.0	61.1 ± 17.9	71.9 ± 16.0	<0.001
Creatinine (mg/dL)	0.86 ± 0.23	0.95 ± 0.29	0.82 ± 0.19	0.014
Creatinine clearance (mL/min)	81.3 ± 29.6	66.0 ± 31.0	88.4 ± 26.1	<0.001
CLCr < 50 mL/min (n[%])	21 (15%)	16 (36%)	5 (5%)	<0.001
P-glycoprotein inhibitor (n[%])	15 (11%)	8 (18%)	7 (7%)	0.060
Estimated Concentrations of plasma rivaroxaban (ng/mL)	192 (85-284)	177 (78-273)	204 (98-290)	0.300

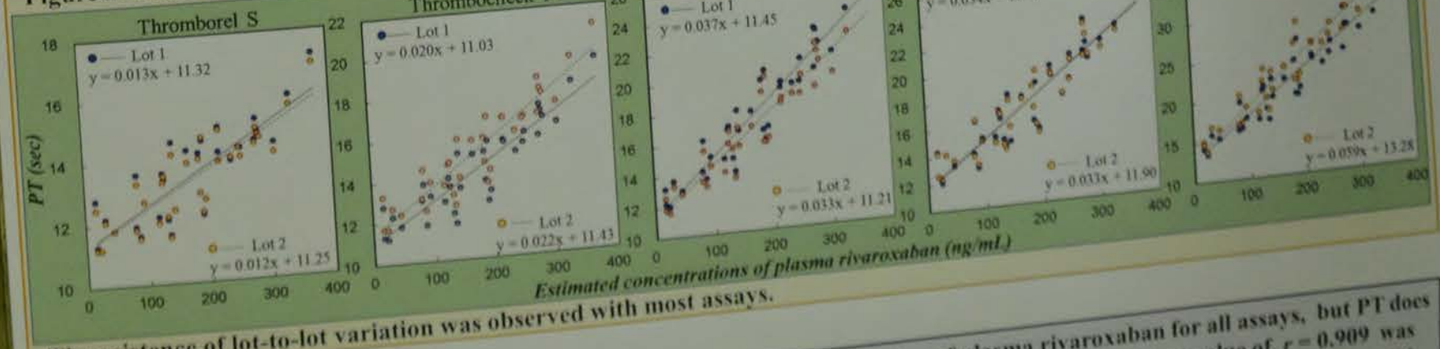
The median estimated concentration of rivaroxaban was 192 ng/mL. 39 samples(28.1%) in the range 0 – 99 ng/mL, 32 samples(23.0%) in the range 100 – 199 ng/mL, 46 samples(33.1%) in the range 200 – 299 ng/mL, 18 samples(12.9%) in the range 300 -399 ng/mL, four samples(2.9%) in the range 400 - 499 ng/mL.

Figure 1. Relationship between the estimated concentration of plasma rivaroxaban and PT measured by five different PT assays. The r value is the correlation coefficient and the SD value is the standard deviation of the regression line.



The correlation corfficient (r) between PT and the estimated concentrations rivaroxaban was as follows : Thromborel S, $r = 0.768$; Thrombocheck, PT, $r = 0.861$; Coagpia PT-N, $r = 0.909$; Neoplastin Plus, $r = 0.882$; Triniclot PT Excel S, $r = 0.870$. A significant linear relationship ($p<0.001$) was observed between PT and the estimated concentrations rivaroxaban for each assay. In contrast, significant difference ($p<0.001$) for all were observed in PT when one PT assay was compared with another. Furthermore, the gradient of the regression lines plotted varied from 0.010 to 0.046, a more then four-fold difference between the shallowest and steepest. The standard deviation of the regression line ranged from 1.001 to 2.980, which tended to be higher for the assays with the higher regression slope gradients.

Figure 2. Comparison of the correlation of estimated concentration of plasma rivaroxaban with PT of two lot (34 samples).



The existence of lot-to-lot variation was observed with most assays.

Discussion

We found significant linear relationship between PT and estimated concentration of plasma rivaroxaban for all assays, but PT does not always predictably reflect the anticoagulant activity of rivaroxaban. The highest correlation coefficient value of $r = 0.909$ was observed with the Coagpia PT-N assay, however, there was a wide range of regression slope gradients ranging more than four-fold from 0.010 for Thromborel S to 0.046 for Triniclot Excel S. This suggests that monitoring PT without a full understanding of the influence of rivaroxaban on each PT assay may lead to clinical error and places patients at risk of bleeding or thrombosis. Furthermore, there was also substantial lot-to-lot variation with some assay. It is essential that when the pharmacological effect of rivaroxaban is evaluated using PT, clinicians should appreciate the difference in sensitivities of assay to rivaroxaban and lot-to-lot variation. In contrast, a study investigating four assays for measuring estimated concentrations of rivaroxaban and lot-to-lot activity reported that each assay showed strong correlation with rivaroxaban concentration, and that the estimated concentrations of rivaroxaban calculated from the anti-Xa activity of the four assays were close. A measurement of the estimated concentrations of rivaroxaban using the anti-Xa activity is not currently performed in the daily clinical practice, but if this technique was adopted as routine, any differences between institutions would be eliminated and the safe use of rivaroxaban would be promoted.

Conclusion

The result of this study showed that there are large differences in PT measured by different assays in the presence of rivaroxaban. When assessing PT to monitor rivaroxaban therapy, clinicians should be aware of interassay and lot-to-lot variation.

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Hematology PC-41

Effects of direct oral anticoa... on general clotting time ass...

Hematology

PC-41

Effects of direct oral anticoagulants on general clotting time assay results

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Introduction and Aim

Direct oral anticoagulants (DOACs) have been developed for prophylaxis and treatment of thromboembolic disorders. When utilized, laboratory monitoring is not necessary, though their effects on clotting assay results are not well understood. We investigated the effects of dabigatran, rivaroxaban, and apixaban on the results of clotting assays used in general examinations.

Objects

Each of examined DOAC was separately added to 4 normal plasma samples (2 commercially available normal plasma samples, homemade pooled normal plasma from 6 healthy subjects, plasma from a healthy donor) at concentrations of 100 and 400 ng/ml.

Methods

- Prothrombin time (PT) was measured with Coapresta® 2000 (SEKISUI Medical, Tokyo, Japan) by using Thrombocheck PT Plus (Sysmex, Kobe, Japan).
- Activated partial thromboplastin time (APTT) was measured with Coapresta® 2000 (SEKISUI Medical, Tokyo, Japan) by using Thrombocheck APTT SLA (Sysmex, Kobe, Japan).
- Fibrinogen (FBG) was measured with Cluss method by using Thrombocheck Fib (L) (Sysmex, Kobe, Japan).Clotting time was measured with Coapresta® 2000 (SEKISUI Medical, Tokyo, Japan) .
- Antithrombin activity (AT) was measured with factor Xa-based chromogenic substrate assays using Test-team® S AT III (SEKISUI Medical, Tokyo, Japan). These results were analyzed by Coapresta® 2000 (SEKISUI, Tokyo, Japan).
- Protein C and protein S were measured with APTT-based assays using STA-Staclot® protein C or S kits (Roche Diagnostic, Tokyo, Japan). These results were analyzed by STA-R® (Roche Diagnostic, Tokyo, Japan).
- Coagulation factor II, V, and VII levels were measured by a one-stage PT-based clotting assay. Coagulation factor VIII and IX levels were measured by an APTT-based clotting assay on the Coapresta® 2000 (SEKISUI Medical, Tokyo, Japan) .
- Statistical analysis : Mann-Whitney Test, Wilcoxon signed-ranks test. Statistical significance was determined with P value < 0.05.

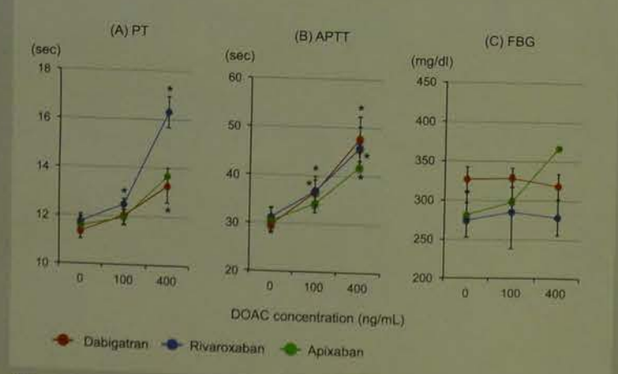

Figure.1
Result of the coagulation screening test.
(A) PT, (B) APTT, (C) FBG.
Data are expressed as the mean ± Standard deviation (N = 4).
* P < 0.05. vs 0 ng/ml

Conclusion

We found that the tested DOACs have varied influence in a concentration-dependent manner on the results of clotting assays used in general examinations. Apixaban tended to exert an overall lower level of influence as compared to dabigatran and rivaroxaban.

Results

- PT and APTT were prolonged by the addition of dabigatran and rivaroxaban in a concentration-dependent manner, while there was no such effect with apixaban. FBG was not influenced by any of the DOACs. (Figure 1).
- Each of the tested DOACs exerted influence on the activities of all examined coagulation factors, except for factor II (Figure 2).
- PS were significantly affected by dabigatran in a concentration-dependent manner, and AT was affected by rivaroxaban. The influence of apixaban on PC, PS, and AT was lower as compared to dabigatran and rivaroxaban (Figure 3).

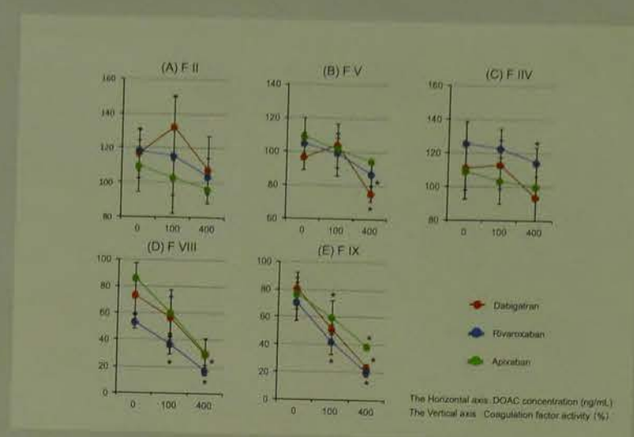

Figure.2
Result of the coagulation factor activities.
(A) Factor II, (B) Factor V, (C) Factor VII, (D) Factor VIII, (E) Factor IX. Data are expressed as the mean ± Standard deviation (N = 4).
* P < 0.05. vs 0 ng/ml

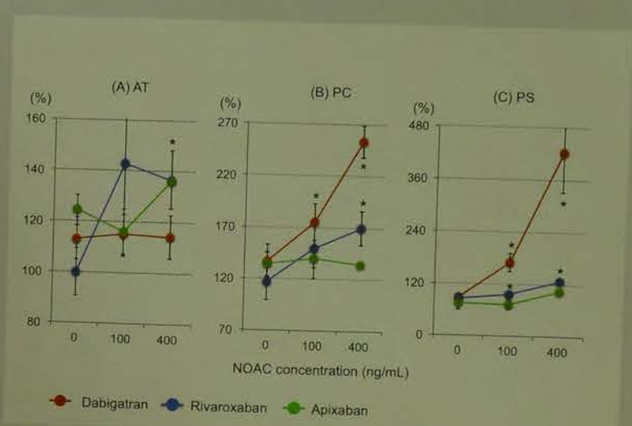

Figure.3
Result of the anticoagulant proteins.
(A) Antithrombin, (B) Protein C, (C) Protein S.
Data are expressed as the mean ± Standard deviation (N = 4).
* P < 0.05. vs 0 ng/ml

Table 1 Effects of DOACs on general clotting time assay results

	DOACs	Dabigatran	Rivaroxaban	Apixaban	
	Factor to receive the inhibition	thrombin	Xa	Xa	effects
Coagulation Screening test	PT	◎	◎	—	prolonged
	APTT	◎	◎	○	prolonged
	Fbg	—	—	—	—
anticoagulant proteins	AT	—	○	—	increase
	PC	◎	○	—	increase
	PS	◎	◎	○	increase
Coagulation Factor Activity (Extrinsic･Common)	F II	△	△	△	—
	F V	○	○	△	decrease
	F VII	△	○	△	decrease
Coagulation Factor Activity (Intrinsic)	F VIII	△	◎	△	decrease
	F IX	◎	◎	○	decrease

◎ strong influence, ○ influence, △ weak influence,
— It doesn't influence.

Disclosure of Conflict of Interest
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PC-42

Laboratory Monitoring of Oral Anti-factor Xa Anticoagulants: Rivaroxaban, Apixaban and Edoxaban

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Background

Direct oral anticoagulants (DOACs) have recently become available for prevention of ischemic stroke in non-valvular atrial fibrillation (NVAF). Sensitive monitoring methods for DOAC therapy are required in some situations. Rivaroxaban, apixaban and edoxaban have been approved as direct oral anti-factor Xa anticoagulants. Here, we investigated the utility of chromogenic anti-Xa assay and standard clotting time assays (PT and APTT) for monitoring of anticoagulant therapy with each anti-Xa anticoagulants using plasma samples from NVAF patients.

Direct oral anti-Xa anticoagulants

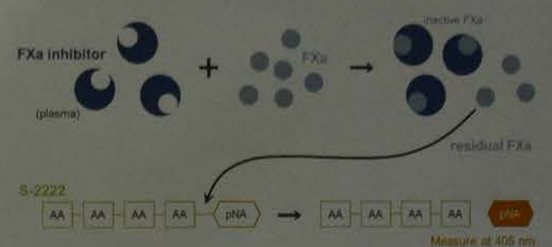
	rivaroxaban	apixaban	edoxaban
Bioavailability	80%	50%	50%
Renal excreation	65% (active form: 36%)	27%	50%
Protein binding	92-95%	87%	40-55%
T_{max}	2-4 hr	3-4 hr	1-2 hr
$T_{1/2}$	5-9 hr	8-15 hr	10-14 hr
Dosage	15 mg qd	5 mg bid	BW > 60 kg: 60 mg qd BW ≤ 60 kg: 30 mg qd

Materials and Methods

Samples

Citrated plasma collected from NVAF patients treated with rivaroxaban, apixaban or edoxaban in Hokko Memorial Clinic (Sapporo, Japan)

The principle of chromogenic anti-Xa assay using S-2222



Calibrated chromogenic anti-Xa assay

Chromogenic substrate: S-2222 (Sekisui Medical)
Calibrators:
Rivaroxban: Biophen Rivaroxaban Plasma Calibrator (Hyphen Biomed)
Apixaban: TECHNOVIEW Apixaban Calibrator (Techno Clone)
Edoxaban: edoxaban-spiked control plasma (edoxaban; Daiichi Sankyo, control plasma; Kyowa Medex)
Analyzer: Hitachi 7170 automatic chemistry analyzer (Hitachi High-Technologies)

Clotting time assays

PT using 3 reagents: Thromborel S (Sysmex), Coagpia PT-N (Sekisui Medical) and Simplastin Excel S (Kyowa Medex)
APTT using 2 reagents: Thrombocheck APTT-SLA (Sysmex) and Platelin L II (Kyowa Medex)
Analyzer: COAGTRON-180 (Kyowa Medex)

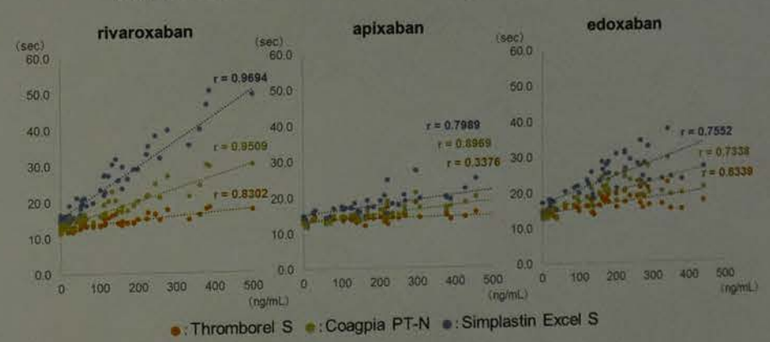
*This study was approved by The Institutional Review Board of Hokkaido University Hospital and Hokko Memorial Clinic.

Results

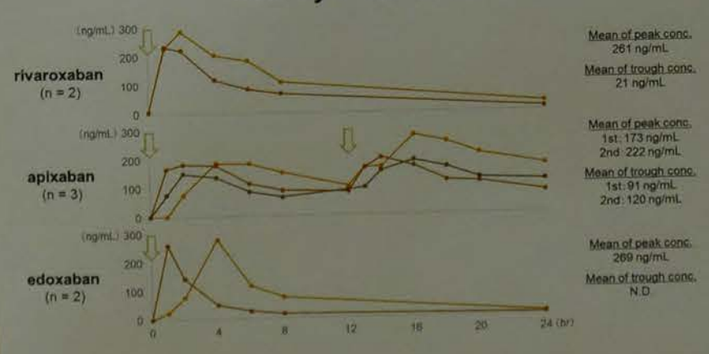
Basic performances of chromogenic anti-Xa assays

		rivaroxaban	apixaban	edoxaban
Repeatability (CV)	Low conc.	2.90%	0.64%	1.47%
	High conc.	0.41%	0.39%	0.50%
Detectable sensitivity		10 ng/mL	20 ng/mL	10 ng/mL
Linearity		~ 500 ng/mL	~ 400 ng/mL	~ 600 ng/mL

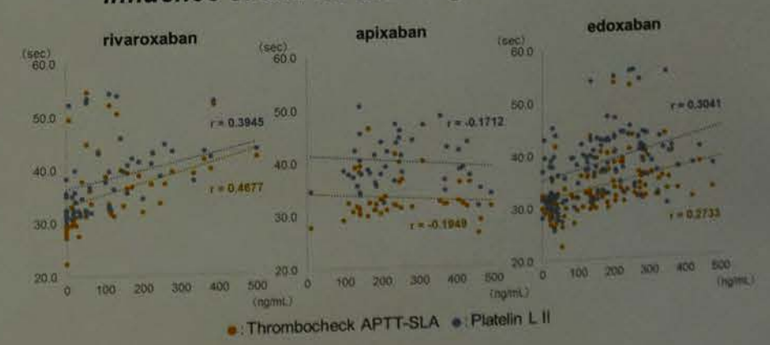
Influence of anti-Xa anticoagulants on PT



Time courses of plasma drug concentrations in healthy volunteers



Influence of anti-Xa anticoagulants on APTT



The present study showed that:
(1) Basic performance of calibrated chromogenic assay using S-2222 was sufficient to determine plasma anti-Xa anticoagulants concentration.
(2) Rivaroxaban and edoxaban induced prolongation of PT in a concentration-dependent manner.
(3) The prolongation of PT induced by rivaroxaban or edoxaban varied widely depending on PT reagents.
(4) Correlations between plasma concentrations of rivaroxaban or edoxaban and APTT were weaker than those for PT.
(5) With regard to apixaban, PT and APTT was inadequate for monitoring of apixaban therapy because of its low sensitivity for plasma apixaban concentrations.

Discussion and Conclusion

This study showed that the chromogenic anti-Xa assay closely reflects plasma anti-Xa anticoagulants concentration, and could be an effective method to assess anticoagulation activity in patients treated with oral anti-Xa anticoagulants. In particular, measurement of plasma drug concentration appears to be useful for patients with bleeding risks such as overdosing, before surgery and renal dysfunction. At the same, PT, which reflects both plasma drug concentration and potential coagulability, should be measured for more practical monitoring of anti-Xa anticoagul... However, it should be considered that the prolongation of PT due to anticoagulation activity of rivaroxaban or edo... varied widely depending on PT reagents. Further investigations for standardization of PT reagents are required. In addition, it also should be considered that measurement of plasma drug concentration is influenced by the timing of blood collection because it fluctuates widely from administration to trough. These findings will contribute to the establishment of a means to monitor anti-Xa anticoagulants treatment.

Hematology

PC-43

Usefulness of clot waveform analysis on the identification of cause for APTT-prolongation

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Introduction

Prolongation of the activated partial thromboplastin time (APTT) may have multiple causes: factor deficiency (FD), inhibition of coagulation factor (FI), lupus anticoagulant (LA), or anticoagulants such as warfarin (War), unfractionated heparin (hep) and direct oral anticoagulants (DOACs). The APTT value alone, though, is incapable of determining the root cause.

It was reported that the ACL-TOP coagulation analyzer can detect LA or FD by mean of the abnormal waveform curves following an APTT assay (*Figure 1*).

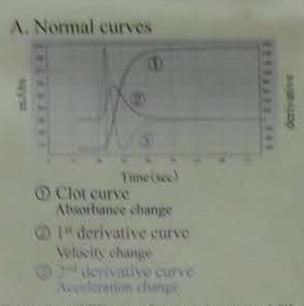


Figure 1. APTT waveform patterns on ACL-TOP coagulation analyzer

Aim

We tried to identify the cause of APTT-prolongation by using numerical clot waveform parameters of the 2nd derivative curve derived from APTT assay on an ACL-TOP system.

Materials and Methods

- Samples

We used the plasma of 36 healthy volunteers (Normal), 24 patients with FD (FVIII:16, FIX:2, FXI:1, FXII:1,FV:1, vWD:3), 6 patients with FI (FVIII:5, FV:1), 61 patients with LA (positive of dRVVT and/or SCT), and 341 patients treated with anticoagulants including War (n = 115), Hep (n = 75), dabigatran (n = 71), rivaroxaban (n = 80).

- Methods (*Figure 2*)

STEP 1; The APTT was measured in all samples and the 2nd derivative curve pattern was analyzed (①,②) .

STEP 2; The numerical waveform analysis parameters (③,④) were calculated.

STEP 3; The sensitivity and specificity for detection of each case were determined using results ①-④ .

STEP 4; The 2nd derivative peak value (⑤) was compared for the different cases of LA, FD and FI. Moreover, the relationship between the 2nd derivative peak and factor VIII activity was studied.

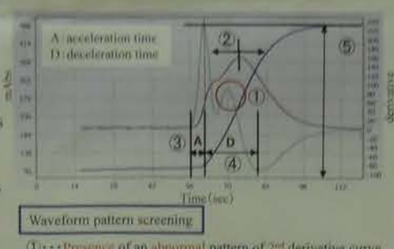
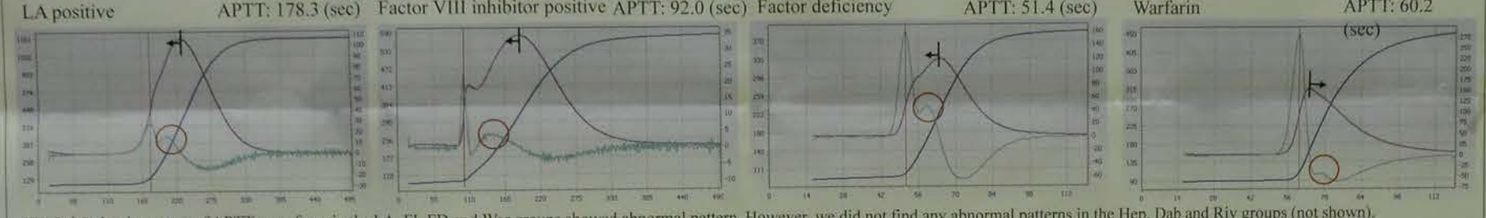


Figure 2. Analysis protocol for APTT waveform

APTT measurement is performed using the APTT-SP reagent (Instrumentation Laboratory) and the ACL-TOP500 coagulation analyzer (Instrumentation Laboratory).

Results

Results 1. Typical abnormal waveform patterns



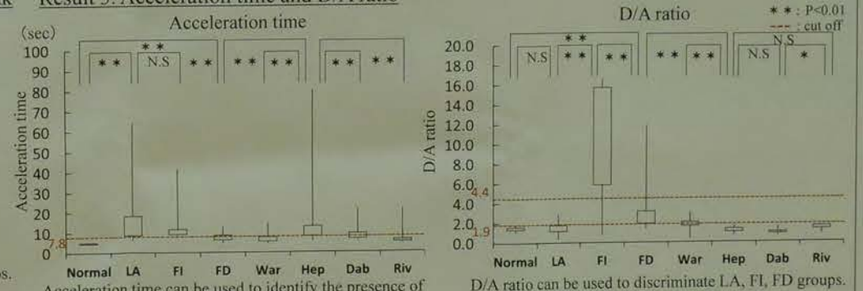
The 2nd derivative curve of APTT waveform in the LA, FI, FD and War groups showed abnormal pattern. However, we did not find any abnormal patterns in the Hep, Dab and Riv groups (not shown). Moreover, the position of the abnormal peak (red circle) was in front of the 1st derivative peak in the LA, FI and FD groups, while it was at the back of the 1st derivative peak in the War group.

Result 2. Frequency of abnormal curve and position of abnormal peak

case	number	APTT (sec) (Mean±SD)	frequency of abnormal curve (%)	position of abnormal curve
Nomal	36	31.8±2.8	0	-
LA	61	61.9±24.4	95.1	Front
FI	6	101.4±52.0	83.3	Front
FD	24	54.7±16.4	95.8	Front
War	115	42.9±7.3	91.3	Back
Hep	75	62.7±37.6	0	-
Dab	71	56.7±18.4	0	-
Riv	80	44.5±6.3	0	-

We detected the abnormal curve with high probability in the LA, FI, FD and War groups.

Result 3. Acceleration time and D/A ratio



Acceleration time can be used to identify the presence of an inhibitor.

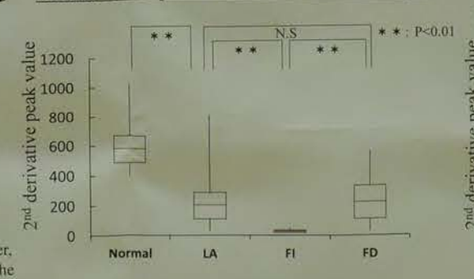
D/A ratio can be used to discriminate LA, FI, FD groups.

Result 4. Detection rate for APTT-prolongation root cause screening

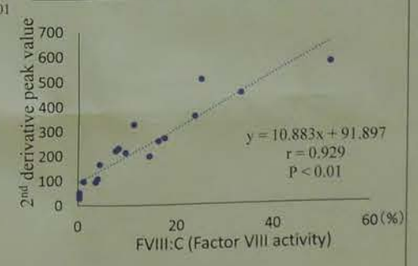
Sensitivity, Specificity (%)		LA	FI	FD	War	Hep	Dab	Riv
waveform	sensitivity	94.5			91.3	100		
	specificity	100			100	92.7		
AT	sensitivity	88.5	100	66.7	80	82.7	59.2	92.5
	specificity	63.9	57.3	44.1	52.1	64.7	44	51.7
D/A	sensitivity	85.2	83.3	75	53.9	100	100	98.8
	specificity	23.2	78.9	81.1	89,6	26.6	26.3	26.7
waveform + AT + D/A	sensitivity	98.4	100	91.7	90.4	100	100	100
	specificity	64.7	56.3	58.6	72.6	53.5	52.9	54.3

Both sensitivity and specificity determined using waveform screening are good. However, the waveform pattern alone cannot discriminate between LA, FI and FD groups. Using the technique in conjugation with numerical waveform parameters, we can detect each case with high sensitivity.

Result 5. Comparison of the 2nd derivative peak



Result 6. Relationship between the value of the 2nd derivative peak and FVIII:C (n =19)



Summary of Results

- We detected abnormal curves in 95.1% of LA group, 83.3% of FI group, 95.8% of FD group and 91.3% of War group. However, we did not find any abnormal curves in patients under the effect of the other anticoagulants (*See Result 1, 2*).
- All abnormal curves in the LA, FI and FD groups were in front of the 1st derivative peak. On the other hand, the abnormal peaks of the War group were located at the back of the 1st derivative peak (*See Result 1, 2*).
- The different cases can be identified using the AT and D/A ratio parameters from the waveform analysis. These parameters along with the waveform pattern, can detect each case with high sensitivity (*See Result 3, 4*).
- The 2nd derivative peak in the FI group was lower than that in the FD and LA groups ($P < 0.01$). In patients with factor VIII deficiency, the 2nd derivative peak correlated with factor activity (*See Result 5, 6*)

Conclusion

- APTT waveform patterns can identify either clinical conditions or anticoagulants with a relatively high probability.
- Both the acceleration time and D/A ratio derived from APTT assay can be used to identify the different cases. We also suppose that using the 2nd derivative peak value, the severity of each case can be determined.
- These waveform analyses can help us in the differential diagnosis of APTT-prolongation.

Clot waveform analysis of the 2nd derivative curve derived from an APTT assay might be useful to identify the cause of APTT-prolongation.

Tokushima University Hospital

Hematology

PC-44

Clot waveform analysis detects very low levels of factor VIII with severe hemophilia A

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2. Dept. Pediatrics, Nara Med. Univ.
3. Sysmex Corporation

Background

Hemophilia A (HA) results from a deficiency of the procoagulant protein factor VIII (FVIII) and is the most common of the severe, inherited bleeding disorders.

A recently developed method to assess comprehensive coagulation function, clot waveform analysis (CWA), can accurately detect low levels (<1 IU/dl) of FVIII activity (FVIII:C) in patients with hemophilia A. (Shima M, et,al. *Thromb Haemost.* 87(3): 436-441. 2002)

Aim

We have attempted to differentiate very low levels of FVIII:C by using CWA.

Methods

- rFVIII was a generous gift from Bayer Corp. Japan. FVIII-deficient plasma (George King), Thrombocheck APTT-SLA (Sysmex), ActinFS and Actin FSL (SIEMENS) were purchased from indicated venders.
- FVIII:C was measured by one-stage clotting assay.
- Patient's plasmas was analyzed by APTT-based CWA by CS-2000i (Sysmex).

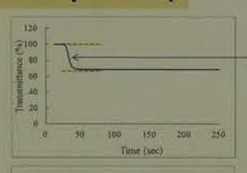
Classification of HA patients

	FVIII:C (IU/dl)	FVIII inhibitor (BU/ml)	Cases
Extremely severe HA (ES-HA)	<0.2	n.d.	10
Very severe HA (VS-HA)	0.2-<0.5	n.d.	12
Modestly severe HA (MS-HA)	0.5-<1.0	n.d.	14
HA with inhibitor (HA-inh)	<0.2	28.5 (2.1-442.0)	20

Results

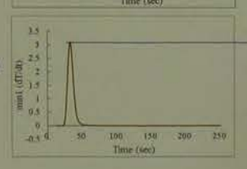
1. CWA (Normal plasma)

Waveform



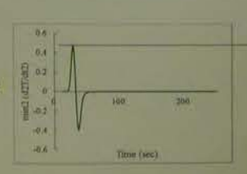
Clot time (CT)

The first derivative curve (dT/dt)



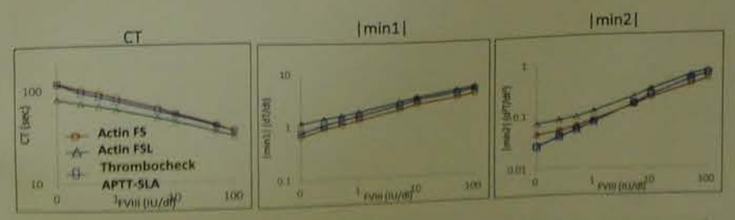
|min1|
(The minimum value of the fist derivative curve ; the maximum velocity of coagulation achieved)

The second derivative curve (d^2T/dt^2)



|min2|
(The minimum value of the second derivative curve ; the maximum coagulation acceleration)

2. CWA of FVIII-deficient plasma mixed with rFVIII



The CT was shorted, and both |min1| and |min2| were increased in a rFVIII dose-dependent fashion, ranging from 0.25-100 IU/dl.
Similar results were observed with each of the APTT reagents.

Table. Intra-assay and inter-assay CV in FVIII-deficient plasmas added various levels of rFVIII

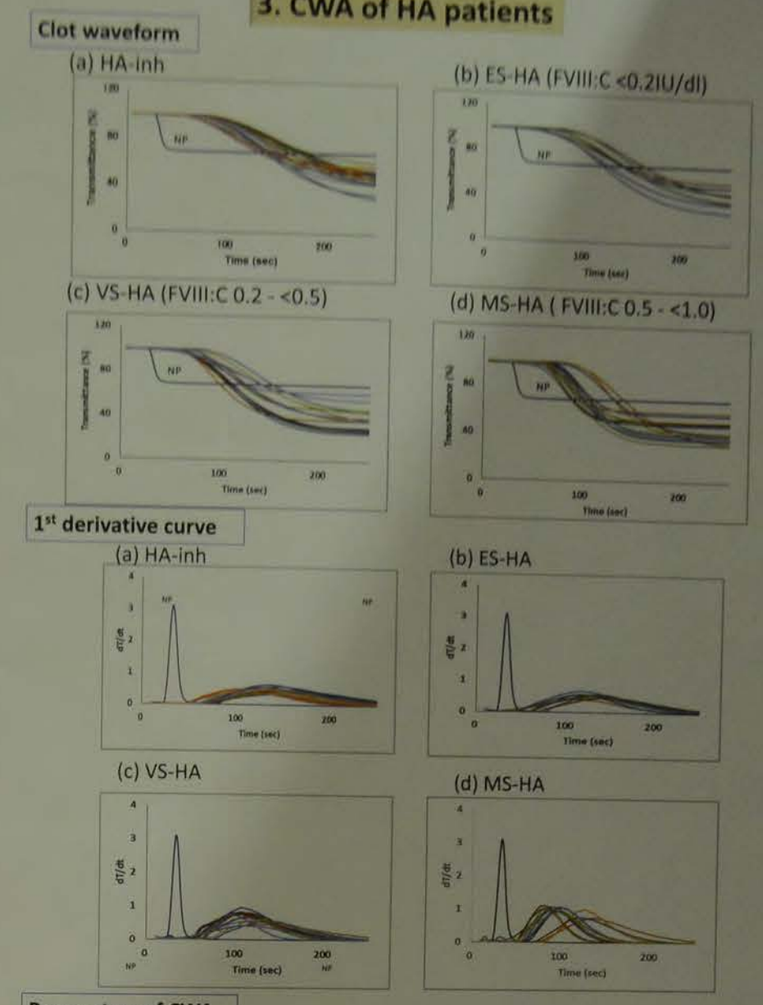
Samples	CT				|min1|				|min2|			
	Intra-assay		Inter-assay		Intra-assay		Inter-assay		Intra-assay		Inter-assay	
FVIII:C (IU/dl)	Mean (sec)	CV (%)	Mean (sec)	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)
100	30.4	0.35	30.6	0.35	4.76	0.40	4.73	0.28	0.739	0.66	0.732	0.48
30	30.9	0.29	39.2	0.19	3.98	0.53	3.97	0.13	0.521	0.64	0.518	0.16
1.0	71.9	0.28	71.7	0.45	1.82	0.72	1.83	0.68	0.106	1.36	0.106	2.38
0.3	86.8	0.30	87.0	0.11	1.36	0.93	1.36	0.36	0.065	1.64	0.064	1.20

The intra-assay CVs were calculated n=10 and the inter-assay CVs were calculated 5daily means/5 results.

The low level of FVIII:C detected appeared to be 0.25 IU/dl.
How about HA patients for coagulation function?

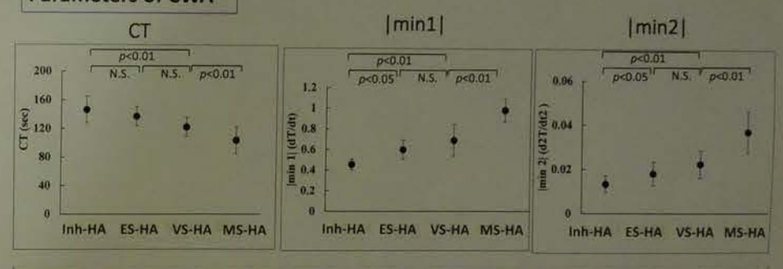
3. CWA of HA patients

Clot waveform



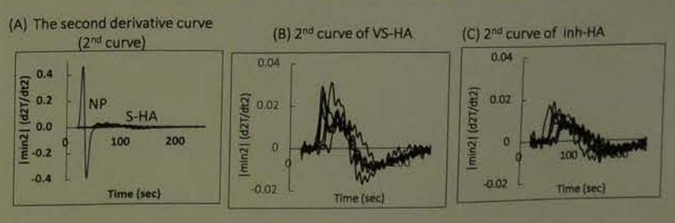
1st derivative curve

Parameters of CWA



The CT was markedly prolonged in all cases but showed little significant differences between the different groups except MS-HA group.
The |min1| and |min2| measurements in HA-inh were lower compared to the other groups.

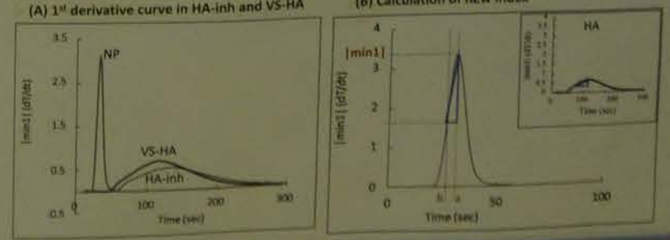
4. Unreliability of |min2|



The findings described above confirmed that |min2| was markedly lower in HA-inh and ES-HA than in NP. The peak levels after the rapid elevation of the first wave appeared consistent but the subsequent decline in slope was irregular and accurate assessments of |min2| at very low levels of FVIII:C in these circumstances were difficult to determine.

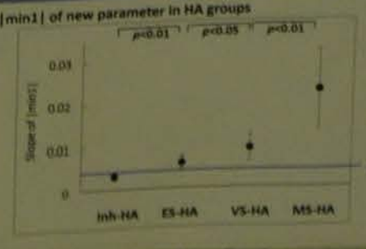
Any parameters that we can classify about presence or absence of FVIII more clearly?

5. The new index parameter and comparisons of slope-|min1| in HA patients.



$$\text{New index} = \frac{1/2 \times |\text{min1}|}{(\text{time to reach to } |\text{min1}|(a) - \text{time to reach to } |\text{min2}|(b))}$$

(C) slope-|min1| of new parameter in HA groups



A new parameter (Slope-|min1|) reflecting average coagulation acceleration was presented.
This index was lower in HA-inh than in VS-HA and ES-HA with greater significant differences ($p<0.01$, <0.05).
The findings indicated that an index of <0.004 reflected the total absence of FVIII in the presence of inhibitor.

Conclusion

The slope-|min1| parameter could provide a useful method for evaluating very low and absent levels of FVIII and/or the development of FVIII inhibitor in HA patients.

COI:
Tomoko Matsumoto
•Course Affiliation: Baxalta Inc.

Hematology

PC-45

A novel diagnostic method APTT clot waveform analysis of coagulation in patients with lupus anticoagulant.

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Toshio Watanabe [1)], Naoki Tokunaga [3)], Keita Morikane [1)]

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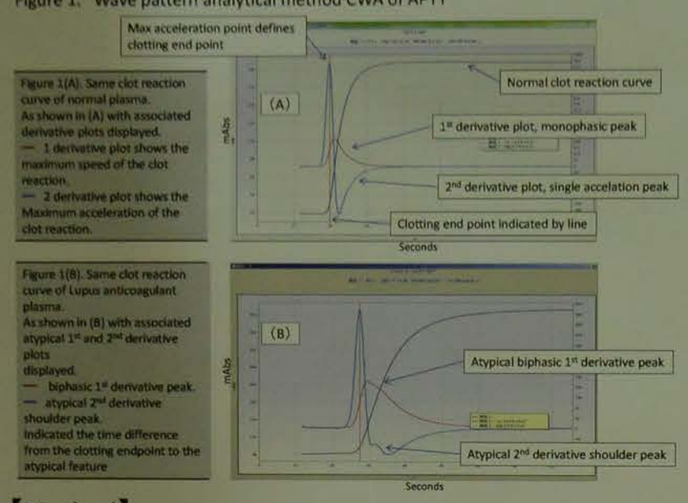
【Introduction】

Measuring activated partial thromboplastin time (APTT) is a standard screening test for coagulation disorders. Next test for the Patients with extended activated partial thromboplastin time (APTT) usually receive the screening tests for lupus anticoagulant (LA), however; such screening tests needs higher cost with the special equipment. We usually experience the abnormal change in the waveform, as which the absorbency change during time course was plotted in the test for APTT. Recently, wave pattern analytical method clot waveform analysis (CWA) was developed, which can be conduct without additional costs. In this study, we investigated the usefulness of CWA using the clinical samples. It was evaluated sensitivity and specificity of LA detected by clinical epidemiologic methods in order to perform the clinical application of CWA.

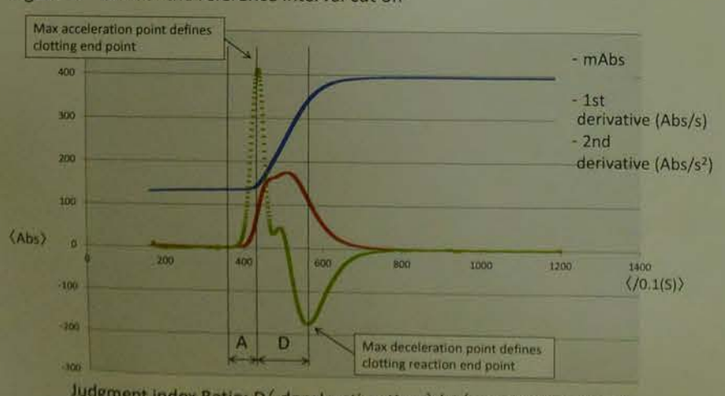
Figure 1. Wave pattern analytical method CWA of APTT



【Method】

A total of 168 samples from 168 patients in whom the presence of LA was measured at Yamagata University Hospital between April 2014 and March 2015. 168 samples were measured by CWA with APTT reagents on the ACL TOP® (IL JAPAN). Blood coagulation waveform was change over time of the quantity of optical transparency to be provided by the fibrin formation, it can be derived acceleration of clotting by second derivative between two points of fixed time. Positive cut-off level（＞2.10）was determined by using the sample form the healthy donor. The presence of LA was confirmed by a positive result in both STACLOT LA® and the dilute Russell viper venom time (DRVVT) test, according to the diagnostic criteria for anti-phospholipid antibody syndrome (APS) which is considered as the gold standard.

Figure 2. CWA of the reference interval cut off



We established cut-off value of Ratio from 20 of normal donors. Cut-off value were derived from the reference interval (RI)mean+ 2 standard deviations(SD) from normal donor populations. This is not inappropriate since date from normal donor populations for Ratio are commonly Gaussian. The upper limit cut off index of Ratio(>2.10) operates as the cut off for determining positivity.

Tokunaga.N et al. TTM Forum13th

【Results】

Positive-LA was presented in 86 of the 168 samples (51.2%). The mean values and standard deviations of APTT seconds values of 86 LA positivity cases were 62.5±25.5, and LA positivity judging were 68 examples among 86 cases , respectively. The mean values and standard deviations of CWA(D/A ratio) of 86 LA positivity cases were 2.23±0.46, and LA positivity judging were 51 examples among 86 cases, respectively. The sensitivity and specificity and Diagnostics accuracy of APTT method were 79.1% and 43.9% and 61.9%, respectively. The sensitivity and specificity of CWA(D/A ratio) method were 59.3% and 85.4% and 72.0%, respectively.

Table 1. LA test results for LA-Positive and LA negative patients used in this study and CWA results

Patients	Number	APTT(S)*	DRVVT ratio*	STACLOT®(S) *	D/A ratio*	D/A ratio pos	APTT(S) Pos
Normal	20	32.5±3.5	1.05±0.1	0.5±2.5	2.00±0.10	0/20	3/20
Warfarin	23	34.0±3.7	1.09±0.1	2.1±1.9	1.98±0.38	11/23	11/23
Coagulation factor deficiency	13	37.0±6.7	1.09±0.1	2.1±1.9	1.84±0.25	1/13	8/13
Heparin	20	42.1±5.3	1.02±0.2	3.6±2.2	1.64±0.29	0/20	19/20
Hemophilia A	3	48.1±9.8	1.01±1.4	4.2±2.6	1.75±0.21	0/3	3/3
Hemophilia B	3	36.5±7.2	1.07±1.2	3.7±1.8	1.91±0.12	0/3	2/3
APS LA pos	86	62.5±25.5	1.65±0.3	27.3±11.3	2.23±0.46	51/86	68/81
Reference range		28.0 - 36.0	< 1.3	< 8.0	< 2.10		

APTT, activated partial thromboplastin time; LA, lupus anticoagulant; DRVVT, dilute Russell viper venom time; APS, antiphospholipid antibody syndrome; D/A, deceleration time/acceleration time ratio ; Warfarin, The oral anticoagulant therapy by warfarin (INR:1.5-3.2); Heparin, The patient in a 5000 units of Heparin(s) medication treatment; STACLOT® , LA measurement kit marketed by Roche ; Reference range, manufacture's cut off value; Normal, normal patients - plasma; * ,mean ± standard deviation; LA Judgment, The presence of LA was confirmed by a positive result in STACLOT LA® or the dilute Russell viper venom time (DRVVT) which is considered as the gold standard;

Table 2. The statistics analysis result of LA using CWA vs APTT(S)

		LA Judgment		
		Positive	Negative	Total
CWA (D/A ratio)	Positive	51	12	63
	negative	35	70	105
Total		86	82	168

		LA Judgment		
		Positive	Negative	Total
APTT(S)	Positive	68	46	114
	negative	18	36	54
Total		86	82	168

LA Judgment, The presence of LA was confirmed by a positive result in STACLOT LA® or the dilute Russell viper venom time (DRVVT) which is considered as the gold standard;
D/A ratio , judging standard cut off (>2.10) , APTT, activated partial thromboplastin time; APTT judging standard cut off (28.0s - 36.0s)
Kappa index(95 % Cis) CWA: 0.453(0.308~0.578), APTT:0.231(0.08~0.379)

Table 3. The statistics analysis result of LA using CWA vs APTT(S)

CWA(D/A ratio)	Point estimates and 95 % Cis（Lower, Upper）
Sensitivity	0.593 (0.482, 0.698)
Specificity	0.854 (0.758, 0.922)
Positive predictive value	0.810 (0.691, 0.898)
Negative predictive value	0.667 (0.568, 0.756)
Diagnostics accuracy	0.72 (0.646, 0.787)

APTT(S)	Point estimates and 95 % Cis（Lower, Upper）
Sensitivity	0.791 (0.69, 0.871)
Specificity	0.439 (0.434, 0.590)
Positive predictive value	0.596 (0.501, 0.687)
Negative predictive value	0.667 (0.525, 0.789)
Diagnostics accuracy	0.619 (0.541, 0.693)

Positive predictive value , True positive/(True positive + false positive);
Negative predictive value, True negative/(True negative + false negative);
Diagnostics accuracy,(True positive + True negative)/Total sample;

【Conclusion】

In this study, it was demonstrated that the specificity and the degree of Diagnostics accuracy of CWA to LA diagnosis was higher than APTT. LA diagnosis of coagulation disorders was possible by the CWA of APTT, with lower cost, which can be conducted in many laboratories.

Hematology

PC-46

Dilute Prothrombin Time Assay To Efficiently Detect Lupus Anticoagulants

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Summary

1. Using diluted Thromborel® S regent, the ratio of dilute prothrombin time(dPT) value at 1:160 dilution to the value without dilution, which we refer here as 'dPT-ratio' (see below), could efficiently discriminate LA from other factors which cause APTT prolongation (Fig. 1).

$$\text{dPT-ratio} = \frac{\text{dPT value at 1:160 dilution}}{\text{dPT value without dilution}}$$

2. The dPT-ratio detected LA in two types of samples as follows (Fig. 1).

i): the samples whose APTT values were not prolonged.

ii): the samples obtained from patients who had received anticoagulant therapy with warfarin.

-Conclusion –

The dPT-ratio obtained from the assay procedure using diluted Thromborel® S may be applicable for LA detection.

The 'dPT-ratio' clearly discriminates LA from other factors which cause APTT prolongation

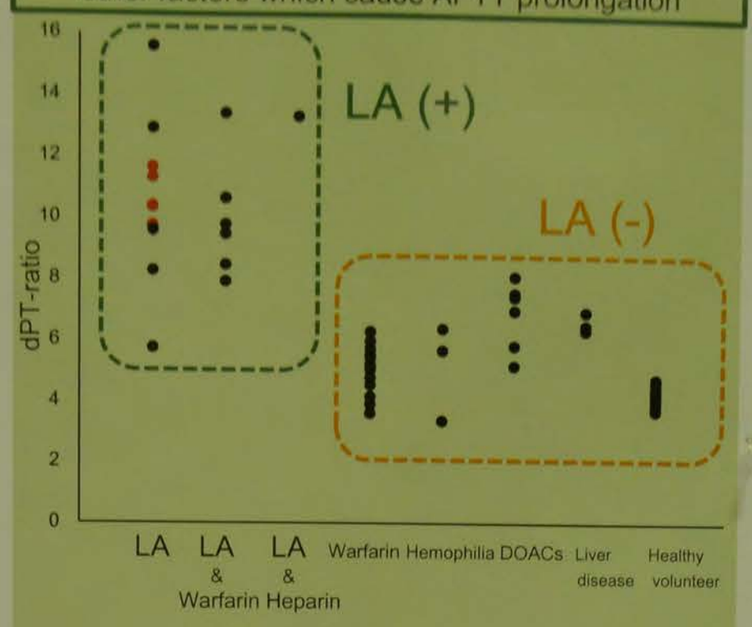

Figure 1. dPT-ratio discriminates samples with LA from those without LA. Red circle(●) represents the samples which did not show APTT prolongation. DOACs: direct oral anticoagulants.

Background and Purpose

Lupus anticoagulants (LA)

- LA are kinds of immunoglobulins which cause prolongation of phospholipid-dependent clotting time[1)].
- LA show close association with recurrent miscarriage, arterial and venous thrombosis[2-3)].

How should clinical laboratories detect LA?

- APTT* and dRVVT** tests are recommended[4-6)].

*APTT: activated partial thromboplastin time, **dRVVT: dilute Russell viper venom time

- APTT and dRVVT, however, show considerable variability in diagnostic performance depending on the reagents used[7)].
- The dPT test is also expected to support diagnosis of LA theoretically[7-9)], but its method has not been established yet.

→ The aim of this study is to establish an efficient procedure of dPT test for LA detection using Thromborel® S, which is a PT reagent widely used in Japan.

Materials and Methods

Materials

PT reagent: Thromborel® S(Siemens, Germany),

samples: LA positive control (IL Japan and PBI, Canada)
FVII, VIII, IX, X deficient plasma (HRF Inc., NC, USA)
Coagtrol 2X (mild multi-factors deficient plasma) (Sysmex, Japan)
Normal plasma (PBI, Canada and healthy volunteer (n=20))

Patients' plasma: LA positive* (n=12), LA positive with warfarin (n=6), LA positive with heparin (n=1), LA negative with warfarin (n=15), Hemophilia (n=3), DOACs (n=7), liver disease (n=3)

*LA positive was confirmed by dRVVT test

Methods

- Lyophilized Thromborel® S was reconstituted with distilled water, and then diluted doubling from 1:10 to 1:320 with 20mmol/L $CaCl_2$
- Employing standard PT assay program, the optimal dilution ratio for the dPT test were determined for various types of samples using automated coagulation analyzer CA-8000(Sysmex, Japan).
- The determined procedure was applied to patients' samples with or without LA.

Results and Discussion

- The prolongation of dPT was observed depending on increasing dilution ratio for all samples (Fig. 2).

→ Considering the performance characteristics for routine purposes, the optimal dilution ratio to distinguish LA from factor deficiency was 1:160.

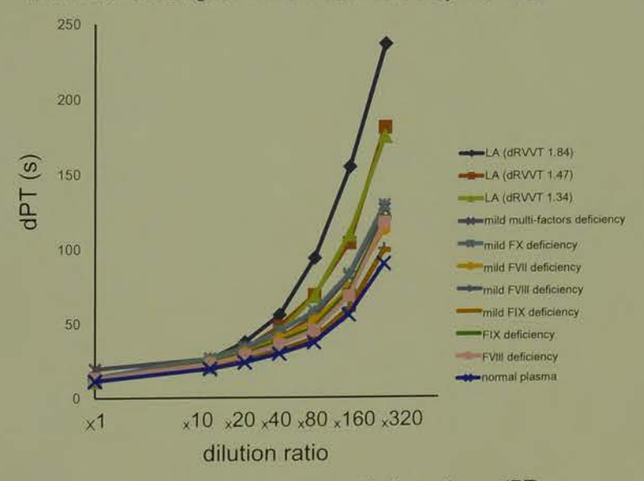

Figure 2. Effects of increasing dilution ratio on dPT

- Heparin-containing and warfarin-containing samples showed extreme dPT prolongation like LA samples (Fig. 3).

→ The former prolongation could be corrected by addition of protamine[10)] (Fig. 3a), the latter was clearly discriminated from LA using the 'dPT-ratio' (Fig. 3b).

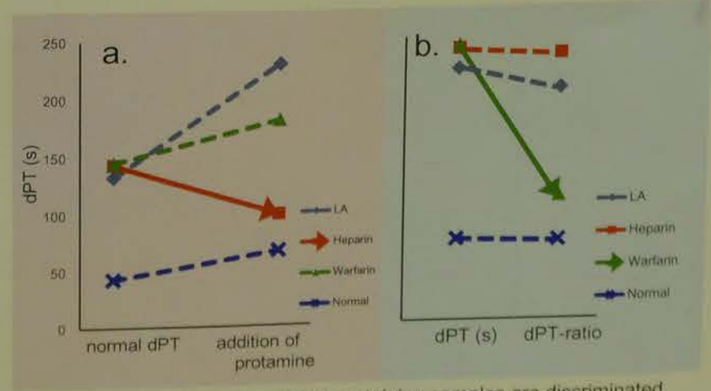

Figure 3. Heparin- and warfarin-containing samples are discriminated from samples with LA using protamine (a.) or dPT-ratio (b.)

- The dPT-ratio detected LA in the samples in which APTT test failed to detect. Moreover, the dPT-ratio could detect LA in warfarin-containing samples (Fig. 1).

→ APTT test has significant variation in the sensitivities to LA depending on the reagents used[11)].

- The lupus ratio test*** has high sensitivity and specificity for LA detection[8,12-13)].

*** the ratio of two clotting times obtained with low and high phospholipid concentrations

- We have no COI to be disclosed. -

Reference

1) Arnout, 2001; 2) Rai et al., 1995; 3) Galli et al., 2003; 4) Pengo et al., 2009; 5) Keeling et al., 2012; 6) CLSI guideline, 2014; 7) Arnout et al., 1994 8) Liestøl et al., 2002; 9) Moore, 2010; 10) Parkin et al., 1949; 11) Brandt et al., 1995; 12) Schjetlein et al., 1993; 13) Jacobsen et al., 2000.

A-4 A-4

Immunology PB-14

PB-13

PB-14

Hematology

PC-47

Usefulness of the platelet parameter: Mean Platelet Volume (MPV) and Mean Platelet Component (MPC) obtained by the ADVIA2120 as convenient indicator for MDS diagnosis.

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INTRODUCTION

It is well known that degranulated platelets or giant platelets may appear in the peripheral blood of the patent with myelodysplastic syndrome (MDS). In general, diagnostic criteria based on morphological characteristics have a defect in its reproducibility because of a wide variability among observers.
Thus, those morphological abnormalities of platelets may be difficult to use as indicators to discriminate MDS from other hematological abnormalities. Here, we report that the mean platelet component concentration (MPC) and the mean platelet volume (MPV), which can be obtained by the ADVIA2120i (Siemens Inc.), are useful and convenient indicators for MDS diagnosis.

MATERIALS AND METHODS

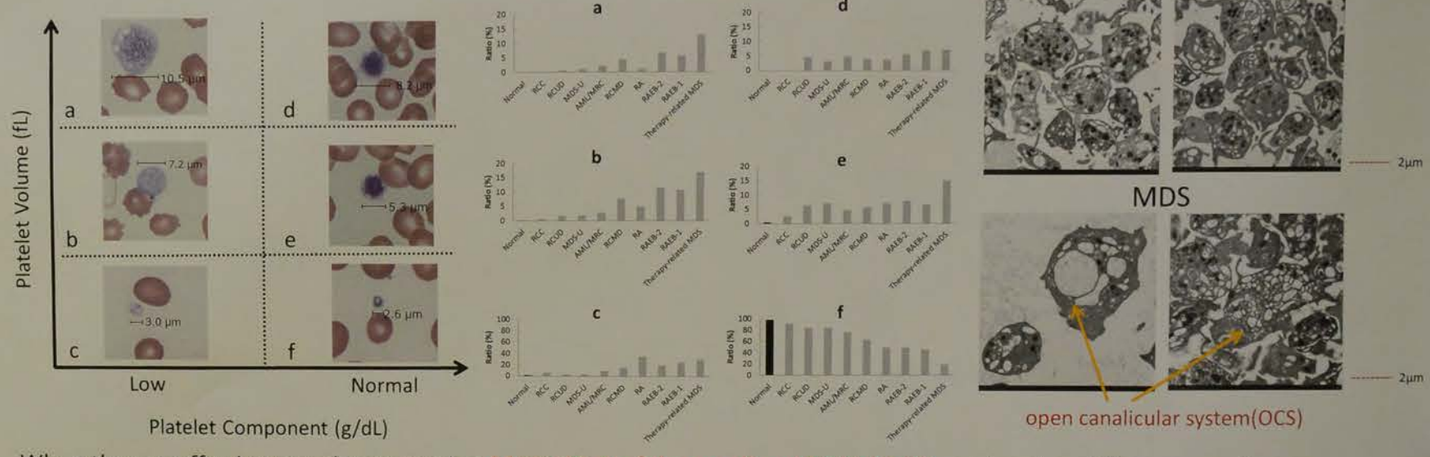
MPC and MPV of platelets in the peripheral blood obtained from 38 cases with MDS, as well as those obtained from 1256 healthy controls, were analyzed, and the cut-off value to discriminate the MDS group from the healthy control was determined. Healthy control and MDS peripheral blood smear of the sample after performing the May-Giemsa staining, 100 of the size of platelets were measured by phase-contrast microscope GX-710 (KEYENCE, Osaka, Japan) .
100 pieces platelets a visually classified into six patterns (in combination as the size of the platelet normal (and 3 patterns of <4μm), large (4 -8μm), giant (> 8μm), and normal or low degree of platelet azure granules) and it was compared classification results of the healthy control and MDS sample that was classified according to WHO 2008 classification of MDS.
In addition, degranulation of platelets in patients with MDS was observed using an electron microscope.

RESULT

The MPC of MDS patients (median 23.0g/dL) was significantly decreased compared with that of healthy controls (median 26.5g/dL). In smear specimens of the peripheral blood, degranulation of platelets was detected in MDS patients.
The MPV of MDS patients (median 10.7fL) was significantly elevated compared with that of healthy controls (median 7.8fL).

groups	healthy control	MDS
n	1256	38
age	64 (1-93)	74 (2-94)
Male/Female	443/813	11/27
PLT ($x10^9$/L)	222 (162-329)	62 (6-542)
MPV (fL)	7.8 (6.3-11.1)	10.7 (7.8-17.7)
MPC (g/dL)	26.5 (19.7-30.0)	23.0 (20.6-29.8)
MPM (pg)	1.97 (1.47-2.74)	2.26 (1.83-3.19)
PDW (%)	48.6 (33.6-70.0)	67.2 (17.0-76.2)

In smear specimens stained by May-Grunwald Giemsa staining, giant and degranulated platelets were detected in MDS patients. Furthermore, platelet of MDS patient had large and/or many open canalicular system (OCS) and low number of α granule compared to healthy control observed by electron microscope.

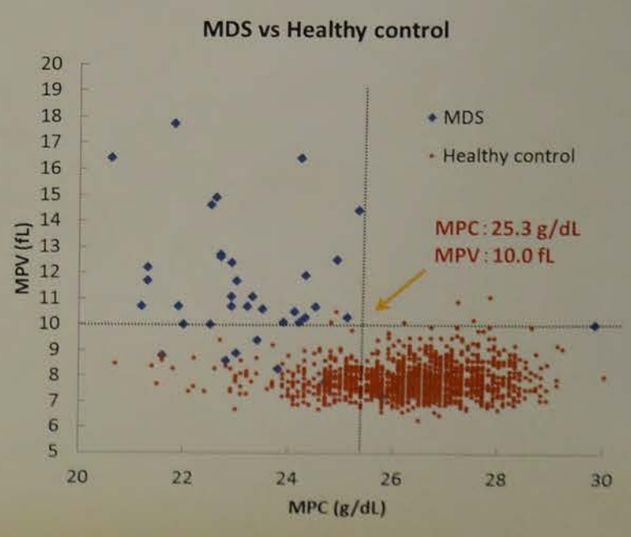


When the cut-off value was determined as **MPC <25.3 g/dL as well as MPV >10.0fL**, each value of the area under curve, sensitivity, specificity, positive predictive value and negative predictive value showed 0.920, 78.9%, 99.9%, 96.8% and 99.4%, respectively.

Discussion

・In this study, we showed the usefulness of the MPC and the MPV, which can be obtained by the ADVIA2120i for MDS diagnosis. Since platelet parameters are affected by mechanical calibration, it is important that fine-tune setting should be required at each facility.

・From observation with optical and electron microscopy, MPV was reflected the size of platelets objectively and MPC reflected the internal components of the platelets objectively . Large platelet with poor (scarce) azurophill granue appears in the peripheral blood of MDS patients. These platelets were thought to be platelets having released alpha granules into the blood, by increasing the open canalicular system (OCS) .Thus low MPC value may mean the reduced internal protein component of the platelets and/or increased OCS.



Hematology

PC-48

Influence of heat shock protein 72 on platelet aggregation in rodents

HSP72 promotes platelet aggregation in the presence of various platelet activators

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Background

Blood Heat shock protein 72 (HSP72) levels positively correlate with increased numbers of clots in coronary arteries of patients with acute myocardial infarction (MI). The clinical significance of elevated HSP72 levels is not well understood; however, platelet aggregation is known to contribute to the formation of thrombi in ruptured plaques during MI.

This study aimed to understand the role of HSP72 in thrombosis by analyzing the effect of HSP72 on platelet aggregation.

Methods

Rat platelet aggregation *in vitro*

Blood was collected from the heart of adult male (13-week old) Sprague-Dawley rats (SLC, Hamamatsu, Japan), in 1/10 volume of 3.13 mol/L sodium citrate and used for the preparation of platelet-rich plasma (PRP).

Aggregation studies were carried out with PRP (4.0×10^6 platelets/ml) using an optical aggregometer (Tokyokoden, Tokyo, Japan). PRP was equilibrated at 37 ℃ for 1 min under constant stirring (1,000 rpm) before aggregation was induced. Final concentration of adenosine diphosphate (ADP; Sigma, MO, USA) used to induce aggregation was 1 μmol/L or 10 μmol/L with 0-10 μg/mL HSP72 (ENZO Lifesciences, NY, USA). The other aggregation activators with 10 μg/mL HSP72 were used final concentration of 0.5 μg/ml collagen (MC medical, Tokyo, Japan), 50 μmol/L thrombin receptor activating peptide-6 (TRAP-6; Sigma, MO, USA), 1 mg/mL ristocetin (MP biomedicals, CA, USA) or 200 μmol/L arachidonic acid (AA; Sigma, MO, USA).

Changes in aggregation were estimated by simultaneous addition of 1 μmol/L ADP, 10 μg/mL HSP72, with or without 2.5 μg/ml anti-HSP72 antibody. Aggregation was monitored as changes in light transmission for 6 min at 37 ℃.

Presence of HSP72 in hyperlipidemia mice *in vivo*

Male (five-week-old) C57BL/6J mice, and ApoE- deficient mice (C57BL/6.KOR/StmSlc-Apoe^{shl}) developing hyperlipidemia, were purchased from SLC (Hamamatsu, Japan). Serum of ApoE-deficient 12-week-old male mice fed a high-fat diet was collected and total cholesterol (T-CHO), triglycerides (TGs) and HSP72 was determined. Immunohistochemistry was performed for HSP72 presence in formalin-fixed paraffin-embedded heart coronary arteries tissue sections.

Statistical analysis of data

Statistical significance was evaluated using Dunnett's multiple comparison test with JMP12 software.

Results

Effect of co-addition of HSP72 in the presence of ADP on the rat platelet aggregation *in vitro*

We investigated the influence of HSP72 on the platelet aggregation in rat PRP induced by ADP. Addition of HSP72 up to 10 μg/mL gave no significant change in the extent of platelet aggregation when PRP were incubated with 10 μmol/L ADP (Fig. 1). On the other hand, the rate of platelet aggregation increased by dose dependent manner of HSP72 concentration, when PRP were incubated with 1 μmol/L ADP (Fig. 2). Moreover, the addition of higher than 4 μg/mL of HSP72 achieved significant acceleration in platelet aggregation compared with that induced by 1 μmol/L of ADP ($p < 0.05$, Fig. 2).

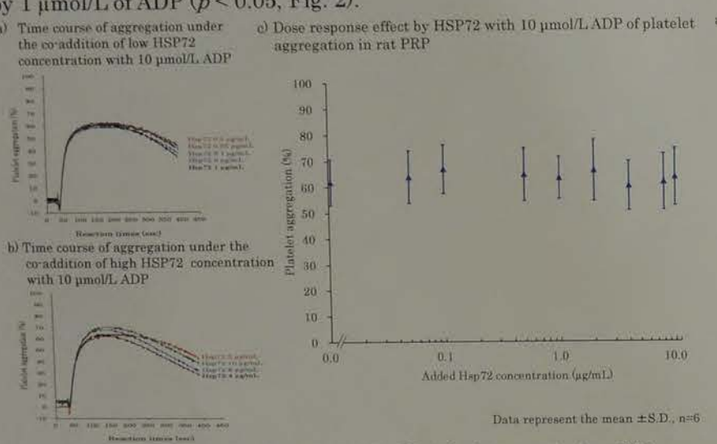

Fig.1. Activation by co-addition of HSP72 with 10 μmol/L ADP of platelet aggregation in rat PRP

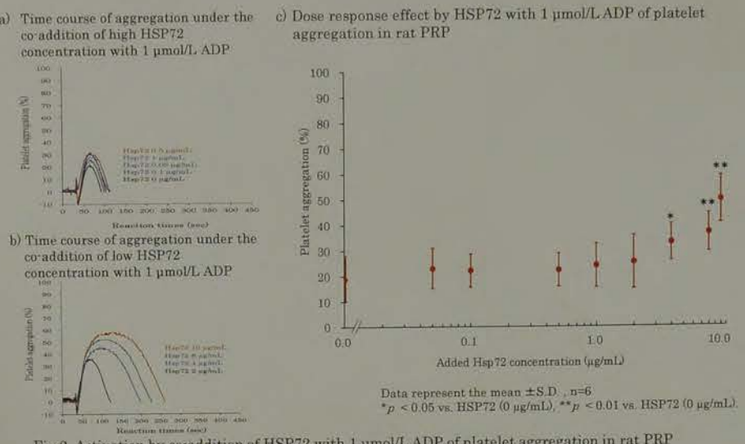

Fig.2. Activation by co-addition of HSP72 with 1 μmol/L ADP of platelet aggregation in rat PRP

Effect of co-addition of HSP72 under the presence of low-concentration of collagen, TRAP-6, ristocetin and AA on the rat platelet aggregation *in vitro*

To define further effect of HSP72, the addition of HSP72 was examined on the platelet aggregation under the presence of 0.5 μg/ml collagen, 50 μmol/L TRAP-6, 1 mg/mL ristocetin or 200 μmol/L AA (Fig. 3).

Significant acceleration of the platelet aggregation by HSP72 addition was observed under the presence of low-concentration ristocetin ($p < 0.05$, Fig. 3), collagen, TRAP-6, or AA ($p < 0.01$, Fig. 3).

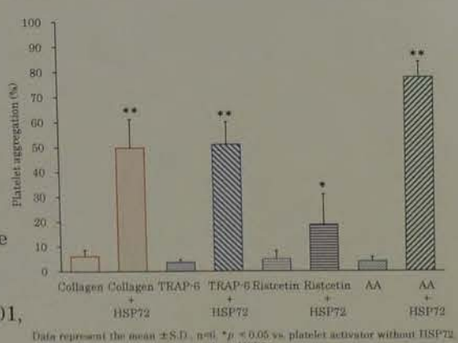

Fig.3. Activation by co-addition of HSP72 with platelet activators of platelet aggregation in rat PRP

Effect of co-addition of HSP72 and anti-HSP72 antibody under the presence of ADP on the rat platelet aggregation *in vitro*

We examined the inhibitory effect of anti-HSP72 antibody on the platelet aggregation in PRP induced by ADP with the presence of HSP72 (Fig. 4). Addition of 2.5 μg/mL anti-HSP72 antibody significantly reduced the platelet aggregation effect induced by 1μmol/L ADP and 10 μg/mL HSP72 ($p < 0.05$, Fig. 4).

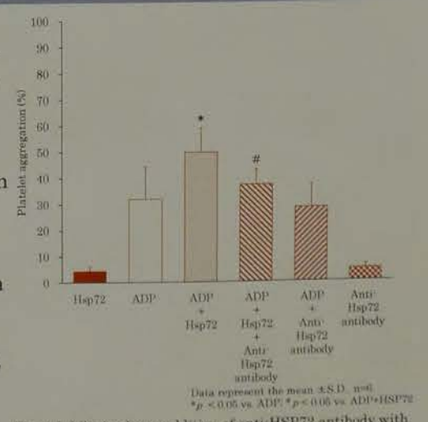

Fig.4. Inhibition by co-addition of anti-HSP72 antibody with HSP72 and ADP of platelet aggregation in rat PRP

Presence of serum HSP72 in hyperlipidemia mice *in vivo*

In our study, we fed ApoE-deficient mice a high-fat diet to induce hyperlipidemia. As a result, T-CHO, TGs, and oxLDL levels were higher than those in normal control ($p < 0.01$, Fig. 5). Additionally, the serum HSP72 of mice fed a high-fat diet increased compared with those of mice fed a normal diet ($p < 0.01$, Fig. 5).

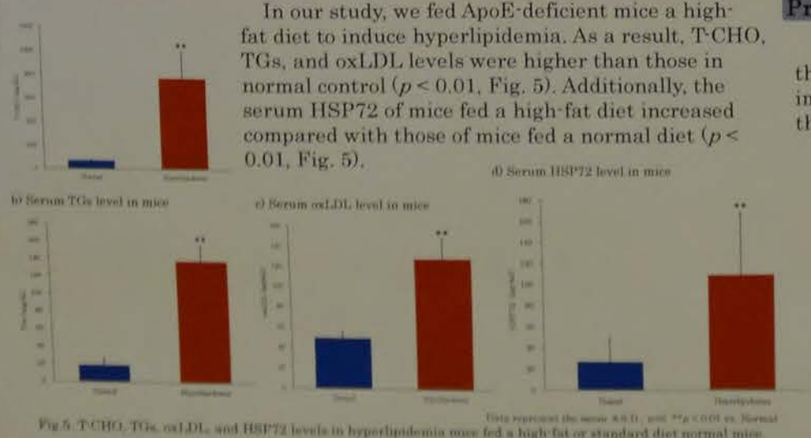

Fig.5. T-CHO, TGs, oxLDL, and HSP72 levels in hyperlipidemia mice fed a high-fat or standard diet normal mice.

Presence of HSP72 on thrombosis in hyperlipidemia mice

In this study, we examined the presence of HSP72 on thrombosis in intravascular of hyperlipidemia mice using immunohistochemistry. Our results demonstrated that thrombosis was dyed with HSP72 antibody (Fig. 6).

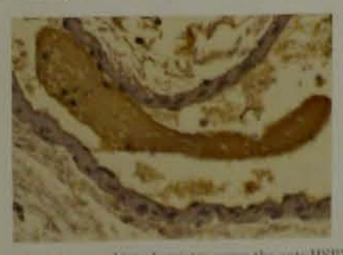

Fig.6. Thrombotic immunohistochemistry using the anti-HSP72 antibody in the blood vessel of hyperlipidemic mouse.

Conclusion

In this study, we investigated the effects of HSP72 on platelet aggregation *in vitro* using rat PRP, induced by a platelet activator such as ADP, collagen, TRAP-6, ristocetin or AA. We demonstrated that HSP72 significantly enhanced the platelet aggregation activated with low concentration of these platelet activators. Moreover, the platelet aggregation induced by HSP72 and ADP was markedly suppressed by further addition of anti-HSP72 antibody. In addition, serum HSP72 increased with a hyperlipidemic mice and, HSP72 was present during thrombosis in hyperlipidemic mice.

We suggest that increased HSP72 in the blood by hyperlipidemia promotes platelet aggregation during formation of thrombi on failed plaques. Thus, HSP72 blood levels may contribute to predict MI.

Hematology

PC-49

A simple method based on peripheral blood parameters for early diagnosis of chronic myelogenous leukemia

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INTRODUCTION

1. Chronic myelogenous leukemia (CML) is a representative disease of myeloproliferative neoplasms (MPN), featured by increase of WBC and platelet counts at the time of onset.
1. The diagnosis of CML essentially requires detection of *BCR-ABL1*. However, its result is not always promptly available.
1. In order to develop a simple and rapid method for the early diagnosis of CML, we analyzed the peripheral blood parameters of the patients.

MATERIALS AND METHODS

Materials: A total of 115 adult CML patients diagnosed at Tokai University Hospital between 2004 and 2016 were enrolled. As a control, 541 adult patients with leucocytosis > 8.0 × 10^9 /L and 193 patients with other types of MPN were included.

Methods:

1. Peripheral blood parameters at the first visit were studied; WBC,platelet counts(PLT), basophil counts (Baso), immature granulocyte counts (IG)(myelocytes and metamyelocytes) and NAP score.
2. A reciever operating characteristic (ROC) curve for each parameter was constructed, and the optimal cut-off value was determined.
3. For NAP score, statistical difference between CML and MPN, and the cut-off value were determined.

IRB approval: Tokai University Hospital (16R019)

RESULTS

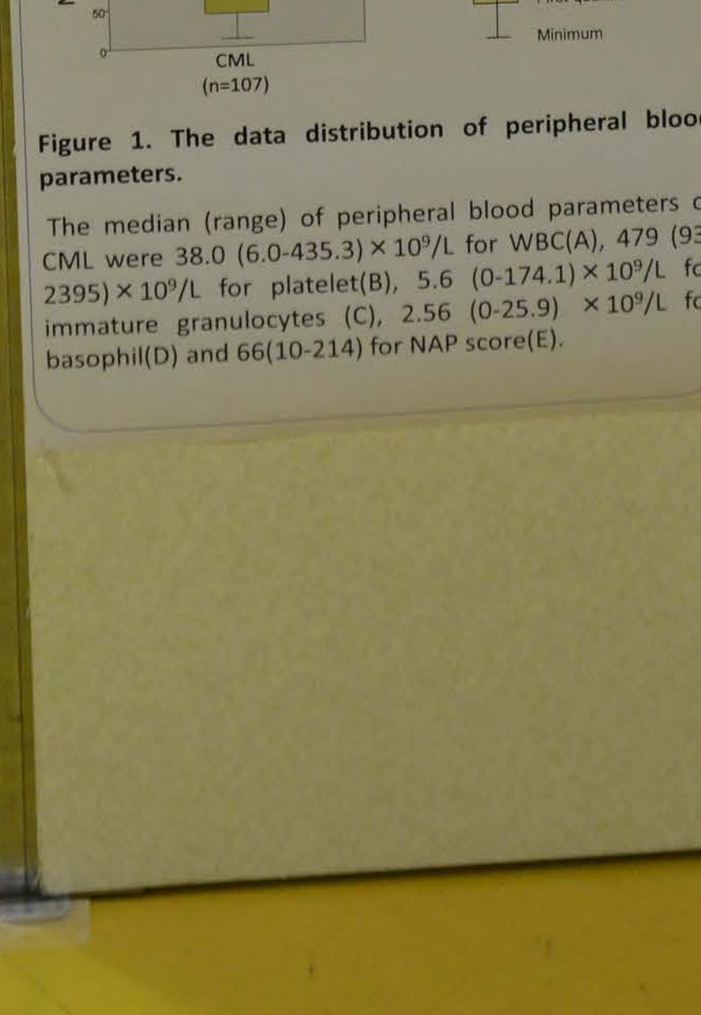
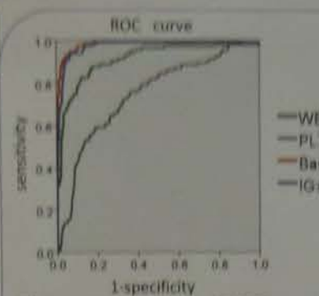


Figure 1. The data distribution of peripheral blood parameters.

The median (range) of peripheral blood parameters of CML were 38.0 (6.0-435.3) × 10^9/L for WBC(A), 479 (93-2395) × 10^9/L for platelet(B), 5.6 (0-174.1) × 10^9/L for immature granulocytes (C), 2.56 (0-25.9) × 10^9/L for basophil(D) and 66(10-214) for NAP score(E).



Parameters	ROC (AUC)	Cut-off value (× 10^9/L)
WBC	0.912	21.0
PLT	0.747	326.0
Baso	0.981	0.43
IG	0.975	0.48

Figure 2. The ROC curve and the cut-off value for peripheral blood parameters

The area under curve of ROC curve was the highest in basophil at 0.981, followed by IG at 0.975, WBC at 0.912 and platelet at 0.747. The cut-off value was 0.43 × 10^9/L, 0.48 × 10^9/L, 21.0 × 10^9/L and 326.0 × 10^9/L, respectively.

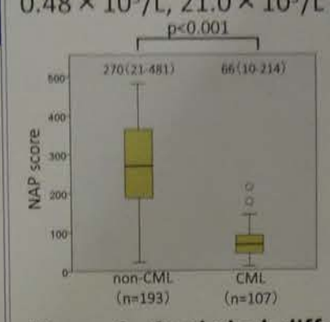


Figure 3. Statistical difference of NAP score between CML and MPN

NAP score was statistically different between CML and MPN (p<0.001)

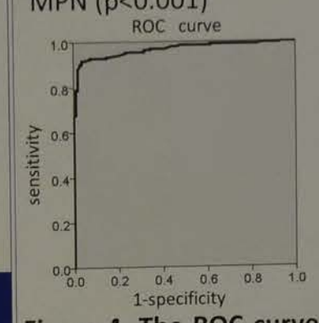


Figure 4. The ROC curve and the cut-off value for NAP score

The are under curve of ROC curve was NAP score at 0.966.The cut-off value was 127.5.

Table 1. Sensitivity and specificity of the basophil count for CML

Overall sensitivity and specificity for CML of the basophil were 93.0% (107/115) and 95.2%(515/541), respectively. Many of the false-positive cases at the time of screening by the basophil, is the other MPN disease type patients.

	Baso# (× 10^9/L) ≧0.43	<0.43
CML (cases)	107	8
non-CML (cases)	26	515

Sensitivity : 93.0%
Specificity : 95.2%

Table 2. Sensitivity and specificity of the NAP score for CML

Sensitivity and specificity for CML of the NAP score were 96.2% (103/107) and 91.7%(177/193), respectively. The false-positive patients at the time of screening by the NAP score was 16 cases. The breakdown were 8 cases of ET,3 cases of PV,2 cases of MF and 3 cases of unspecified MPN.

	NAP score <127.5	≧127.5
CML (cases)	103	4
non-CML (cases)	16	177

Sensitivity : 96.2%
Specificity : 91.7%

CONCLUSION

1. In screening of CML, the absolute number of basophil was the most suitable marker.
2. When the *BCR-ABL1* is not promtly available, early screening of CML and its differentiation from other types of MPN can be made simply by a stepwise use of the absolute number of basophil, followed by NAP score.

Hematology

PC-50

Mean Platelet Volume is a beneficial prognostic biomarker in patients with type 2 diabetes

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Abstract

Background and Objective
Mean Platelet Volume (MPV), the most commonly used measure of platelet size, has recently been reported to be associated with a risk for cardiovascular disease. Then, MPV is thought to be potentially useful as a predictor of cardiovascular risk. Cardiovascular events in patients with type 2 diabetes mellitus may also be associated with accelerated platelet activation shown in high MPV. So, we hypothesized that increased MPV may be related to the pathogenesis of type 2 diabetes and can be a beneficial biomarker for reflecting platelet activation in the setting of diabetic vascular complications.

Methods
We classified the patients into three groups with diagnostic criteria (fasting glucose level and HbA1c) and analyzed MPV: non-diabetic group (Control group), patients with impaired glucose tolerance (pre-DM group), patients with type 2 diabetes (T2DM group).

Results
MPV was significantly increased in T2DM group as compared to Control group. In pre-DM group, MPV was already significantly increased. To determine whether an association between MPV and arteriosclerosis in type 2 diabetes, we next measured Cardio Ankle Vascular Index (CAVI) by using pulse wave velocity and Intima-Media Thickness (IMT) by performing carotid artery echo. Then, T2DM group showed significant increase of CAVI and IMT as compared to Control group. Moreover, We found that MPV is importantly associated with diabetic cardiovascular complication.

Conclusion
It was suggested that platelet activity of diabetic patients become more reactive due to increased MPV. Furthermore, enhanced cardiovascular risk in type 2 diabetes may be a result of high MPV. Therefore, MPV can be a potentially beneficial prognostic biomarker in type 2 diabetes patients.

Introduction

Mean Platelet Volume (MPV), the most commonly used measure of platelet size, is a marker of platelet function and activation. It has been reported that MPV is significantly associated with the setting of cardiovascular disease, so MPV is thought to be potentially useful as a predictor of cardiovascular risk.

Mean platelet volume as a predictor of cardiovascular risk: systematic review and meta-analysis (Chu S.G. et al. *J. Thromb. Haemost.* 2010)

High platelet volume and increased risk of myocardial infarction (Kodvaite J. et al. *J. Thromb. Haemost.* 2011)

·Type 2 diabetes (T2DM) is a metabolic disorder and becomes a crucial health problem in the world. T2DM induces some diabetic cardiovascular complications such as atherosclerosis, myocardial infarction, and so on. Cardiovascular events in patients with type 2 diabetes mellitus may also be associated with accelerated platelet activation shown in high MPV.

·So, we hypothesized that increased MPV may be related to the pathogenesis of type 2 diabetes and can be a beneficial biomarker for reflecting platelet activity in the setting of diabetic vascular complications.

Method

Patient classification
We classified the patients into three groups with diagnostic criteria (fasting glucose level and HbA1c): non-diabetic group (**Control group n=30**), patients with impaired glucose tolerance (**pre-DM group n=30**), patients with type 2 diabetes (**T2DM group n=80**).

- **Control group : Fasting glucose levels ≦110 mg/dl**
- **pre-DM group : HbA1c 5.7～6.4 %, Fasting glucose levels 100 ～ 125 mg/dl**
- **T2DM group : HbA1c ≧6.5 %, Fasting glucose levels ≧126 mg/dl**

(Based on diagnostic criteria of JDS and ADA)

MPV measuring by hematology analyzer 'ADVIA'

MPV was analyzed by automatic blood counter (ADVIA 2120i or ADVIA120 Hematology System, Siemens, Inc.) whose measurement principle was flow cytometry-based analysis of blood.

Results

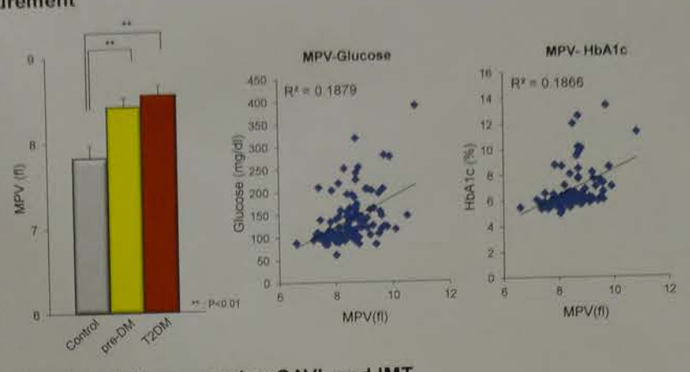
1.Patient characteristics

	Control	pre-DM	T2DM
Age (years)	62.55±4.87	72.33±1.53	72.68±1.40
Weight (kg)	59.44±1.86	62.90±1.93	60.55±2.09
BMI (kg/m^2)	22.33±0.61	23.32±0.56	23.91±0.62
DM duration (years)	-	-	10.06±0.75
Fasting glucose (mg/dl)	94.47±3.94	109.57±1.81	181.80±8.74
HbA1c (%)	5.48±0.12	6.06±0.05	8.21±0.30
TG (mg/dl)	114.91±10.68	159.0±20.65	164.91±17.95
LDL-C (mg/dl)	106.38±8.46	106.23±10.75	107.09±4.45

2. Result of MPV measurement

MPV was significantly increased in T2DM and pre-DM group as compared to Control group. We also found a tendency that fasting glucose level and HbA1c was elevated as MPV was increased.

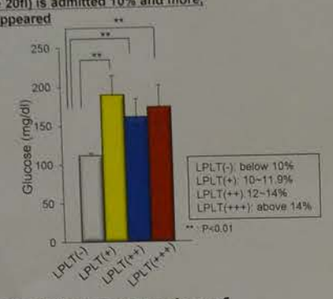
Next, we investigated the relationship between blood glucose and platelet size by morphology flag 'LPLT'. When large platelet (above 20fl) is admitted 10% and more, LPLT flag is appeared. This flag is ADVIA specific.



3. More analysis of platelet-glucose relationship by using morphology flag 'LPLT'

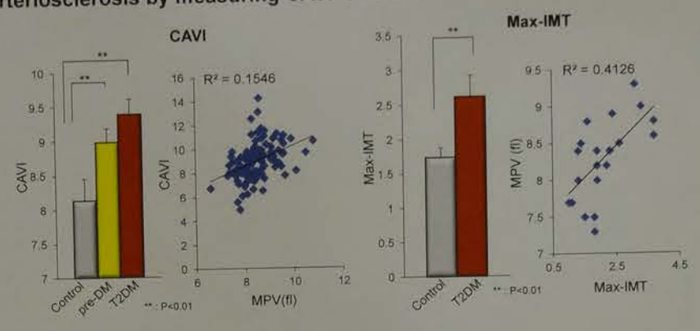
When large platelet (above 20fl) is admitted 10% and more, morphology flag LPLT is appeared

LPLT positive patients (+~+++) showed a significant hyperglycemia as compared to LPLT negative (-) patients. This result suggested that platelet size (MPV) was strongly associated with blood glucose in T2DM patients.



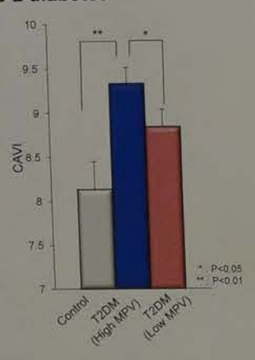
4.The evaluation of arteriosclerosis by measuring CAVI and IMT

To determine whether an association between MPV and arteriosclerosis in T2DM, we next measured Cardio Ankle Vascular Index (CAVI) and Intima-Media Thickness (IMT). These are good markers for evaluating of arteriosclerosis.
T2DM group showed a significant increase of CAVI and IMT as compared to Control. Moreover, MPV showed a positive correlation with CAVI and IMT.



5. High MPV will affect the progression of arteriosclerosis in type 2 diabetes

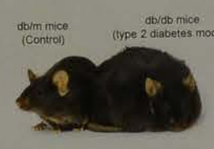
Furthermore, we hypothesized that the progression of arteriosclerosis is not permitted if MPV is not increased.
Because Control group' MPV was 7.8fl, we divided T2DM into 2 groups and analyze CAVI; **high MPV group (MPV>7.8fl)** and **low MPV group (MPV<7.8fl)**.
Then, low MPV group showed a significant decrease of CAVI compared to high MPV group, so MPV is importantly associated with diabetic cardiovascular complications in T2DM.



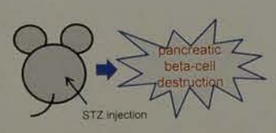
6. Further analysis by using rodent models (db/db mice and STZ induced diabetic mice)

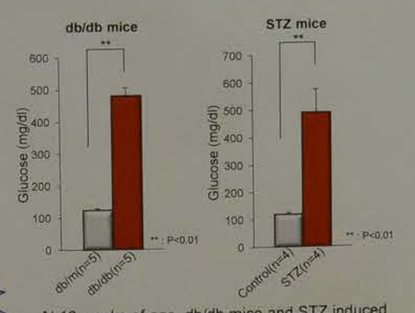
To investigate the mechanism that MPV is increased by diabetic condition, we measured MPV in db/db mice and Streptozotocin (STZ) induced diabetic mice(STZ mice).

·db/db mice is a model of obesity, diabetes wherein *leptin* receptor activity is deficient because the mice are homozygous for a point mutation in the gene for the *leptin* receptor.



·STZ destroys pancreatic beta-cells and STZ induced mice show acute hyperglycemia and hypoinsulinemia.

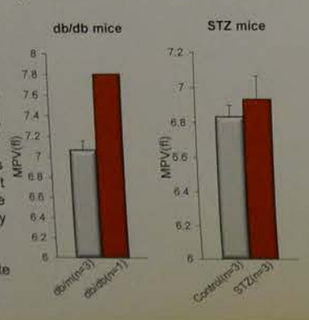




At 10 weeks of age, db/db mice and STZ induced diabetic mice showed a significant hyperglycemia as compared to Control mice.

7. MPV measuring in db/db mice and STZ mice

In db/db mice, we found a tendency that MPV was increased as compared to db/m mice. In STZ mice, however, MPV was not increased. So, it was suggested that MPV may be influenced by chronic hyperglycemia rather than acute one.



Summary

The diabetic condition in T2DM
↓
MPV↑
↓
The progression of arteriosclerosis in T2DM

Conclusion

**We concluded that platelet activation in diabetic patients become more reactive due to increased MPV.
Furthermore, enhanced cardiovascular risk in type 2 diabetes may be a result of high MPV.
Therefore, MPV can be a potentially beneficial prognostic biomarker in type 2 diabetes patients.**

Hematology

PC-51

Immature Reticulocyte Fraction may Predict the Hematopoietic Recovery after Hematopoietic Stem Cell Transplantation

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Abstract

It is important to assess engraftment and hematopoietic recovery after hematopoietic stem cell transplantation. Since immature reticulocyte fraction (IRF) and immature platelet fraction (IPF) indicate hematopoietic recovery, it is possible that IRF and IPF are useful predictors for hematopoietic recovery after hematopoietic stem cell transplantation. Subjects were 25 patients who underwent hematopoietic stem cell transplantation. We measured neutrophil, reticulocyte, platelet, IRF and IPF in peripheral blood sample. The median day of neutrophil engraftment and IRF>10% was shorter than that of reticulocyte and platelet engraftment. IRF was significantly increased on day 15 after hematopoietic stem cell transplantation. Neutrophil was not significantly increased on day 15 after hematopoietic stem cell transplantation. Our results indicated that IRF was a useful predictor and could be incorporated into routine care as novel tool for hematopoietic recovery after hematopoietic stem cell transplantation.

Background

Hematopoietic stem cell transplantation is one of the important therapies in hematopoietic malignancies. It is important and difficult to assess hematopoietic recovery after hematopoietic stem cell transplantation. We must assess engraftment in early for saving the use of granulocyte-colony stimulating factor and blood products.

New indicators of hematopoietic recovery are being studied. These include the immature reticulocyte fraction (IRF) and immature platelet fraction (IPF), which represent hematopoietic function of reticulocytes and platelet, and include higher ribonucleic acid content. So far these fraction has been measured by flow cytometry. In recent years, these fractions can be calculated conveniently and inexpensively by automated hematology analyzer. The objective of our study was to evaluate that IRF and IPF are useful predictors and incorporated into routine care for hematopoietic recovery after hematopoietic stem cell transplantation.

Present criteria of engraftment	
Neutrophil	>0.5 × 10⁹/L
Reticulocyte	>1.0%
Platelet	>20 × 10⁹/L or 50 × 10⁹/L

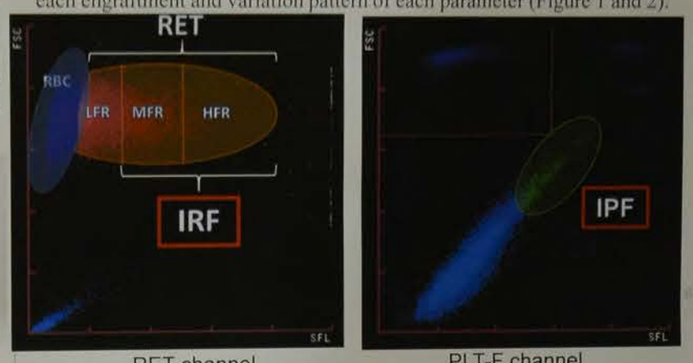
Hematology analyzer XN-1000® (Sysmex)

Methods

Subjects: 25 patients who underwent hematopoietic stem cell transplantation in Tohoku University Hospital from June 2015 to April 2016 (Table 1).

Laboratory data: We measured neutrophil, reticulocyte, platelet, IRF and IPF in peripheral blood sample by automated hematology analyzer XN-1000® (Sysmex, Kobe, Japan).

Statistics analysis: Friedman's test and multiple comparison using Bonferroni correction were used to determined the difference in day of each engraftment and variation pattern of each parameter (Figure 1 and 2).



RET channel PLT-F channel

IRF and IPF were determined by fluorescence and light scatter-based methods, using fluorescent RNA markers that enable distinction between immature and mature cell fractions.

Results and Discussion

Table 1. Characteristics of patients

Sex, n	
Male	13
Female	12
Age, years, median (range)	48 (22-65)
Disease, n	
Acute myeloid leukemia	10
Acute lymphoid leukemia	6
Hodgkin's lymphoma	1
Non-Hodgkin's lymphoma	5
Other	3
Cell source, n	
Allogenic bone marrow	10
Allogenic peripheral blood	7
Autologous peripheral blood	3
Cord blood	5

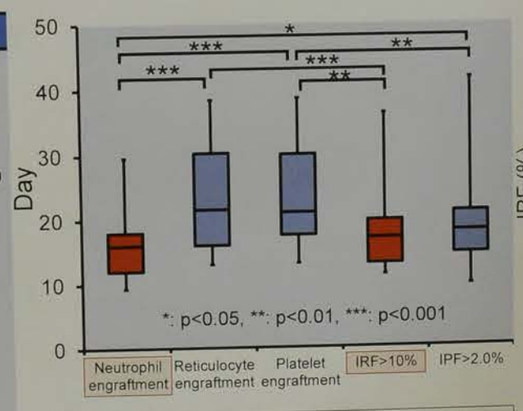


Figure 1. The median day of neutrophil engraftment and IRF>10% was shorter than that of reticulocyte and platelet engraftment.

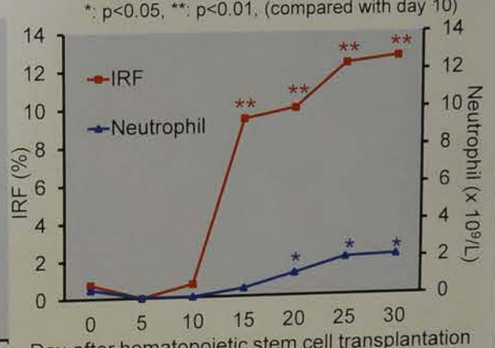


Figure 2. . IRF was significantly increased on day 15 after hematopoietic stem cell transplantation. However, neutrophil was not significantly increased on day 15 after hematopoietic stem cell transplantation.

➢ Change of IRF level was early and remarkable.

Conclusion

IRF is a useful predictor and can be incorporated into routine care as novel tool for hematopoietic recovery after hematopoietic stem cell transplantation.

Hematology

PC-52

Diffuse large B-cell lymphoma (DLBCL)

Introduction

Purpose

Materials and Method

Results

Hematology

PC-52

The serum survivin levels and pathological diagnosis of diffuse large B-cell lymphoma (DLBCL)

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3)Research Institute for Clinical Oncology, Saitama Cancer Center, 4)Pathology, Saitama Cancer Center

Intrduction

What is survivin?

- One of the 8 members of inhibitor-of-apoptosis (IAP) gene family
- 16.5kDa protein (the smallest IAP)
- Localized both inside and outside of the cell (Figure 1)
- Reduces caspase activity, and inhibits apoptosis
- Regulates the cell cycle
- Thought to contribute to cancer cell survival.

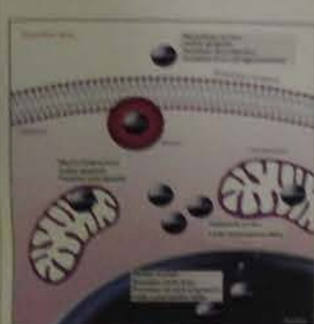

Figure 1. The localization of Survivin.
Survivin is localized both inside and outside of the cell, and exerts different functions. **Extracellular survivin** inhibits apoptosis, and increases cell proliferation. **Cytoplasmic survivin** can't protect the cells from apoptosis. **Mitochondrial survivin** inhibits apoptosis. **Nuclear survivin** regulates cell division.
Dallaglio K, J Invest Dermatol 132, 18–27, 2011

Survivin expression

In tissues

- High level in embryonic tissues
- Low or nondetectable level in normal adult tissues
- Over expression of survivin was found to correlate with drug resistance and poor prognosis in various tumors

In serum

- Some studies indicated high level in various cancers; melanoma, brest cancer, pancreatic cancer, advanced non small cell lung cancer, could be a sensitive marker for metastasis

Diffuse large B-cell lymphoma (DLBCL)

- The most common non-Hodgkin lymphoma, and represents about 30% of malignant lymphomas
- Classified by gene expression profiling (Figure 2) and by immunohistochemical staining (Figure 3,4)
- All three subtypes have different cell origins and therapeutic response
- Some studies indicated that survivin predicted a poor prognosis in patients with DLBCL (Adia C, *et al*. Blood 96: 1921, 2000)

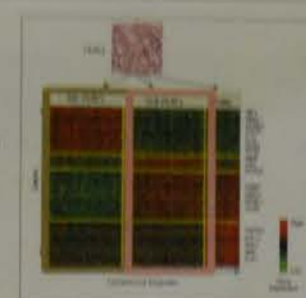

Figure 2. The classification of DLBCL by gene expression profiling. DLBCL can be classified molecularly into 3 subtypes ; activated B-cell (ABC), germinal center B-cell (GCB) and primary mediastinal B-cell.
Dunleavy K, Oncology 28, 326 2014

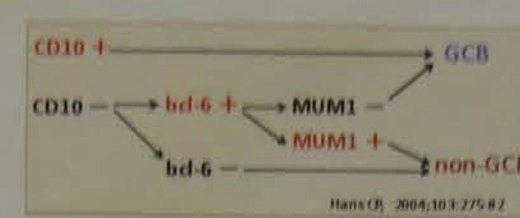

Figure 3. The classification of DLBCL by immunohistochemical staining. Hans algorithm for the determination of molecular DLBCL subtypes is based on the immunohistochemical analysis of three markers (CD10, bcl-6 and MUM1). Using this algorithm, DLBCL can be classified pathologically as germinal center B-cell like (GCB) or non-germinal center B-cell-like (non-GCB).

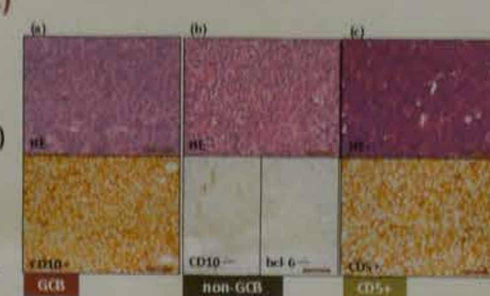

Figure 4. The classification of DLBCL by immunohistochemical staining.
(a) is diagnosed as GCB because CD10 is positive.
(b) is diagnosed as non-GCB because CD10 and bcl-6 are negative. Non-GCB has a poor prognosis than GCB.
In addition, CD5-positive DLBCL is one of the immunohistochemical subgroup in the WHO classification, and known as having a poor prognosis. (c) is an example of CD5-positive DLBCL.

Purpose

The aim of the study is to investigate the relationship between the serum survivin levels and

- Clinical stages
- GCB or non-GCB (by immunohistochemistry, Hans algorithm)
- CD5+ (by immunohistochemistry)
- Other serum biomarkers; LDH and sIL2R

Materials and Method

Materials:

Serum from

- 39 patients diagnosed as DLBCL (15 to 84 year-old)
- 16 healthy adults (23 to 50 year-old) (Table 1,2,3)

Method:

Human Survivin Quantikine ELISA kit (R&D Systems)

Statistical Analyses:

- The best cutoff value that maximizes sensitivity and specificity differentiates DLBCL from controls was calculated by using the ROC (Receiver Operating Characteristic) curves.
- Student's *t*-test and nonparametric Mann-Whitney test were used to compare differences in serum survivin concentrations.

Table 1.
Patients' Clinicopathological characteristics

Pathology		GCB	non-GCB	total
Gender				
	Male	13	12	25
	Female	4	10	14
Age (year)				
	Median	55.5	66.5	61.0
	Range	15-84	19-82	15-84
Originated tumor				
	gastric	2	1	3
	duodenal	1	0	1
	testicular	0	1	1
	other	14	20	34
Stage				
	I	6	3	9
	II	1	6	7
	III	3	4	7
	IV	5	7	12
	Relapse	2	2	4
LDH (U/l)	Median	1198.1	656.9	
	Range	141-3722	142-551	
sIL2R (U/ml)	Median	3682.3	3166.7	
	Range	253-10143	311-4651	
Survivin (pg/ml)	Median	121	51.8	
	Range	0-858.2	0-146.5	

Table 2. Pathological findings of GCB

Case	Age	Sex	CD5	bcl-6	CD10	MUM1	CD20	Pathology	Stage	Survivin	sIL2R	LDH
1	60	M	−	−	+	−	+	GCB	R	858.2	[illegible]	3722
2	70	F	−	+	+	+	+	GCB	R	64.9	[illegible]	[illegible]
3	78	M	NA	NA	NA	NA	NA	GCB	IV	140.2	[illegible]	[illegible]
4	73	M	−	+	+	−	+	GCB	IV	45.2	[illegible]	[illegible]
5	77	M	−	+	+	+	+	GCB	IV	33.4	[illegible]	[illegible]
6	64	F	−	p+	+	−	+	GCB	IV	31.6	[illegible]	[illegible]
7	40	M	−	w+	+	−	+	GCB	IV	20.6	[illegible]	[illegible]
8	34	F	−	NA	NA	NA	+	GCB	III	0	[illegible]	[illegible]
9	42	M	−	+	+	−	+	GCB	III	0	[illegible]	[illegible]
10	42	M	−	+	+	−	+	GCB	III	0	[illegible]	[illegible]
11	67	F	pw+	+	+	+	+	GCB	II	8	1220	[illegible]
12	63	M	−	−	+	−	+	GCB	I	17	[illegible]	[illegible]
13	60	M	−	+	+	−	+	GCB	I	12	[illegible]	[illegible]
14	63	M	pw+	−	+	+	+	GCB	I	7	[illegible]	[illegible]
15	64	M	−	+	+	+	+	GCB	I	5	[illegible]	[illegible]
16	35	M	−	+	+	+	+	GCB	I	0	[illegible]	[illegible]
17	48	M	−	+	+	+	+	GCB	I	0	[illegible]	[illegible]

Table 3. Pathological findings of non GCB

Case	Age	Sex	CD5	bcl-6	CD10	MUM1	CD20	Pathology	Stage	Survivin	sIL2R	LDH
18	42	F	NA	NA	NA	NA	NA	non-GCB	R	43.8	[illegible]	[illegible]
19	64	M	−	+	−	+	+	non-GCB	R	146.5	[illegible]	[illegible]
20	84	F	−	+	−	+	+	non-GCB	IV	106.3	[illegible]	[illegible]
21	66	M	−	−	−	+	+	non-GCB	IV	66.2	[illegible]	[illegible]
22	48	M	−	p+	−	+	+	non-GCB	IV	18.8	[illegible]	[illegible]
23	69	F	−	pw	−	+	+	non-GCB	IV	10.8	[illegible]	401
24	71	F	p+	−	−	+	+	non-GCB	IV	8	[illegible]	[illegible]
25	79	F	−	+	−	+	+	non-GCB	IV	4	[illegible]	[illegible]
26	61	F	NA	NA	NA	NA	NA	non-GCB	IV	1.8	[illegible]	[illegible]
27	73	F	+	+	−	+	+	non-GCB	III	30	751	[illegible]
28	63	M	−	+	−	+	+	non-GCB	III	7.3	[illegible]	[illegible]
29	83	M	−	w+	−	+	+	non-GCB	III	0	[illegible]	[illegible]
30	68	M	−	±	NA	+	+	non-GCB	III	0	[illegible]	[illegible]
31	79	F	+	+	−	+	+	non-GCB	II	53.7	[illegible]	[illegible]
32	43	M	−	+	−	+	+	non-GCB	II	34	[illegible]	[illegible]
33	70	M		−	−	NA	+	non-GCB	II	27	[illegible]	600
34	40	F	−	+	−	+	+	non-GCB	II	0	479	[illegible]
35	44	F	−	+	−	+	+	non-GCB	II	0	[illegible]	[illegible]
36	82	M	−	−	−	NA	+	non-GCB	II	0	[illegible]	[illegible]
37	79	M	−	+	−	+	+	non-GCB	I	4	[illegible]	[illegible]
38	40	M	−	+	−	p+	+	non-GCB	I	0	[illegible]	[illegible]
39	75	M	−	+	−	+	+	non-GCB	I	0	4451	[illegible]

pw; partialweak, p;partial, NA; not available, w; weak

Results

- Healthy adults significantly expressed low serum survivin levels ($p<0.04$; Table 4, Figure 4).
- 4/4 (100%) cases at relapse and 7/12 (58.3%) cases at stage IV were positive for survivin. The positive cases at stage II were non-GCB or CD5+ subtype (Table 4).
- Compared to our previous results of HL and FCL (Fig 5), DLBCL tends to be positive at stage IV and relapse (Table 4,5,6). The serum survivin levels were significantly elevated in patients at stage IV and relapse ($p<0.0005$; Figure 5)
- There was no statistical differences of serum survivin level between GCB cases and non-GCB cases (Figure 6).
- There seemed to be no relationship among the serum survivin, sIL2R and LDH (Figure 7).

Table 4. Summary of serum survivin levels in patients with DLBCL

DLBCL	Positive >20pg/ml	Negative <20pg/ml	Total
Healthy adults	0	16	16
Stage I	0	9	9
Stage II	3	4	7
Stage III	0	7	7
Stage IV	7	5	12
Relapse	4	0	4
Total	14	41	55

Table 5. Serum survivin levels in patients with Hodgikin and Folicular lymphoma.

HL	Positive >20pg/ml	Negative <20pg/ml	Total
Stage I	0	0	0
Stage II	0	3	3
Stage III	0	4	4
Stage IV	0	3	3
Relapse	1	0	1
Total	1	10	11

FCL	Positive >20pg/ml	Negative <20pg/ml	Total
Stage I	0	0	0
Stage II	0	1	1
Stage III	0	2	2
Stage IV	0	5	5
Relapse	2	1	3
Total	2	9	11

Kawamura M, et al 62nd Japanese society of laboratory Medicine Anual meeting, 2015

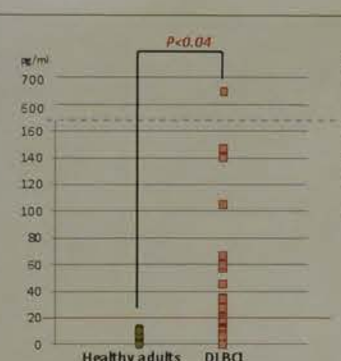

Figure 4. Comparing the serum survivin levels of healthy adults and DLBCL patients. Healthy adults significantly expressed low serum survivin levels ($p<0.04$).

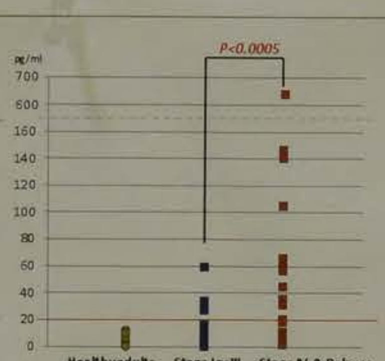

Figure 5. Comparing the serum survivin levels of healthy adults, patients at stage I~III, and patients at stage IV or relapse. The serum survivin levels significantly elevated in patients at stage IV and relapse ($p<0.0005$).

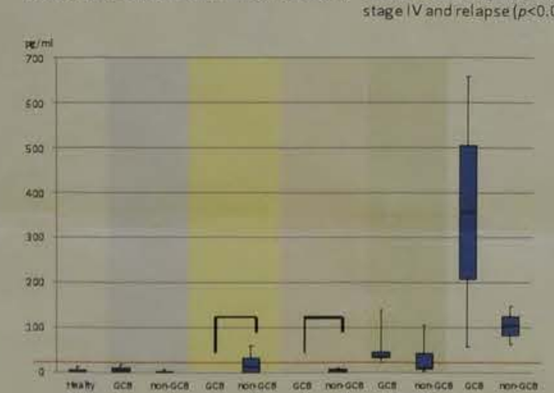

Figure 6. Level of serum survivin GCB vs non GCB at each stages.
At stage II and III, non-GCB cases expressed higher survivin levels than GCB cases. However there was no statistical differences between them. At stage IV and relapse, the survivin levels elevated regardless of GCB or non-GCB.

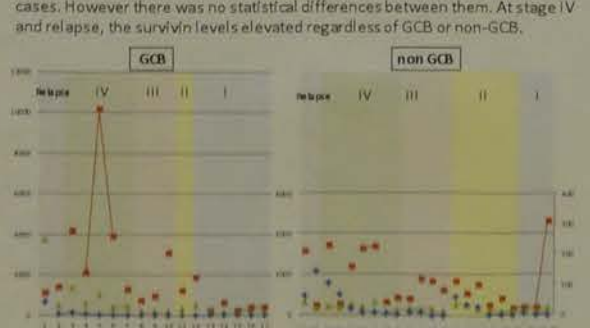

Figure 7. Correlation of survivin, LDH, and sIL2R. There seemed no remarkable correlation among three biomarkers. Even though sIL2R or LDH expressed high level, the survivin level didn't always elevate.

Discussion

- High serum survivin levels might be associated with advanced stages (stage IV) and relapse.
- There was no statistical differences of serum survivin level between GCB cases and non-GCB cases by classification of Hans algorithm. The Hans algorithm based on the expression of three markers was reported a predictivity of 87.3% compared with gene expression profiling . We might need to analyze by gene expression profiling.
- Only one case with CD5+ non-GCB DLBCL case expressed a high survivin level even at stage II.
- Serum survivin levels were not correlated with sIL-2R and LDH.

Conclusions

1. The serum survivin levels could be a biomarker for predicting patients's advanved stage.
2. The serum survivin levels could be a good help for detecting relapse and contribute to early treatment.
3. Further examination is needed to know about correlations between serum survivin and pathological subtypes.
4. Survivin was related to advance/ relapse phase and might be a good target of therapy, such as sepantronium bromide (YM155), selective survivin suppressant.

Acknowledgements

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Conflict of Interest (COI) of the Principal Presenter

No potential COI to disclose

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Hematology

PC-53

Basic evaluation of platelet aggregation measuring system with the fully automated coagulation analyzer

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GIFU UNIVERSITY

Introduction

Platelet aggregation test is an essential method for the diagnostic of patients with platelet function defects and is focused on its usefulness for assessment of antiplatelet therapy in recent years. This test requires specific equipment and manually operation so there is concern over differences between the respective operators or laboratories.

Recently, the fully automated coagulation analyzer CS-series has been upgraded with new software to perform light transmission aggregometry.

In this study, we evaluated the basic performance of the function.

Materials & Methods

We used pooled plasma and plasma of patients treated with antiplatelet agents. 70 individuals were studied in total and the characteristics of them were shown in table 1.

Adjusted platelet-rich plasma (PRP) with a platelet count of 200 x 10^9/L was obtained by diluting PRP with the platelet-poor plasma (PPP).

Platelet aggregation test was performed on coagulation analyzer CS-2400 (Sysmex Corporation, Japan) and MCM HEMA TRACER 712 (MC Medical Inc., Japan; H-TRACER) as a reference instrument. Revohem ADP and Revohem Collagen (Sysmex Corporation, Japan), MCM ADP and MCM Collagen H (Laboratory & Medical Supplies) were used as agonists in CS-2400 and H-TRACER, respectively.

ADP and collagen reagents were prepared to be 30 μM and 5 μg/mL respectively and used for within-run and interference study. For comparison study ADP and collagen reagents were prepared to be 10 μM, 100 μM and 20 μg/mL, 50 μg/mL, respectively and used at each instruments. PRP and agonists were mixed in the ratio of 1:7 in CS-2400 or in the ratio of 1 : 9 in H-TRACER

Within-run precision (n=10) and interference studies were performed in CS-2400. Samples for interference study were prepared by adding interference Check A Plus (SYSMEX Corporation, Japan) to PRP and PPP.

CS-2400

Revohem ADP, Revohem Collagen

Table. 1 Characteristics of the 70 patients

Male / Female	41 / 29
Age (mean ± SD)	68.5 ± 12.64
Antiplatelet agents in taking	
Aspirin	15
Clopidogrel sulfate	17
Cilostazol	5
Aspirin + Clopidogrel sulfate	13
Aspirin + Cilostazol	5
Clopidogrel sulfate + Cilostazol	6
Nothing	9

Results

Table 2 Evaluation of within-run precision by the maximum aggregation in CS-2400

	ADP 30 μM	Collagen 5 μg/mL
Mean ± SD (%)	69.9 ± 4.8	81.2 ± 4.6
CV%	6.79	5.71

The coefficient of variations in within-run imprecision by each agonist showed less than 7%.

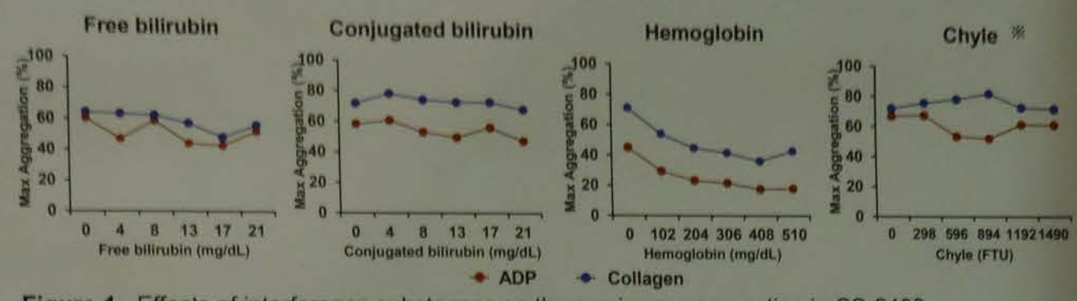


Figure 1 Effects of interference substances on the maximum aggregation in CS-2400
Free bilirubin, conjugated bilirubin, and chyle had few effect on respective platelet aggregation by ADP 30 μM and collagen 5 μg/mL up to the maximum addition concentration, while hemolysis reduced the maximum aggregation by both agonists as its concentration increases.
※Error marks were displayed on the result of chyle.

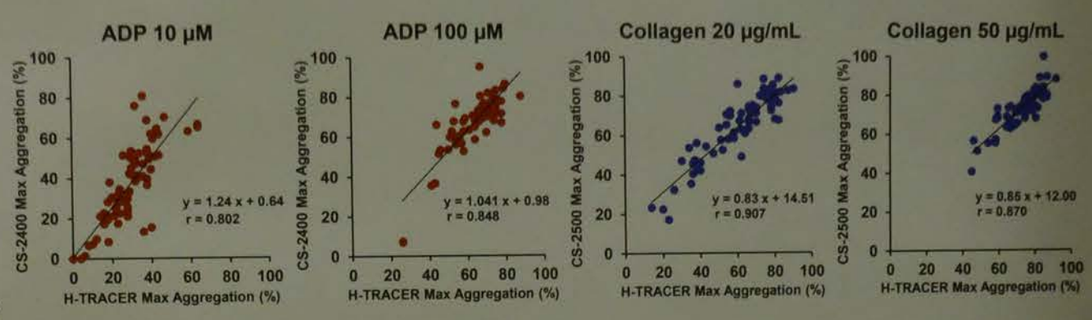


Figure 2 Correlation with the reference method
In comparison study (N = 70), the regression equation and correlation coefficient in ADP 10 μM and 100 μM were Y = 1.24 X + 0.64 and r = 0.802, and Y = 1.04 X + 0.98 and r = 0.848, respectively. Those of collagen 20 μg/mL and 50 μg/mL were Y = 0.83 X + 14.51 and r = 0.907 and Y = 0.85 X + 12.00 and r = 0.870, respectively.

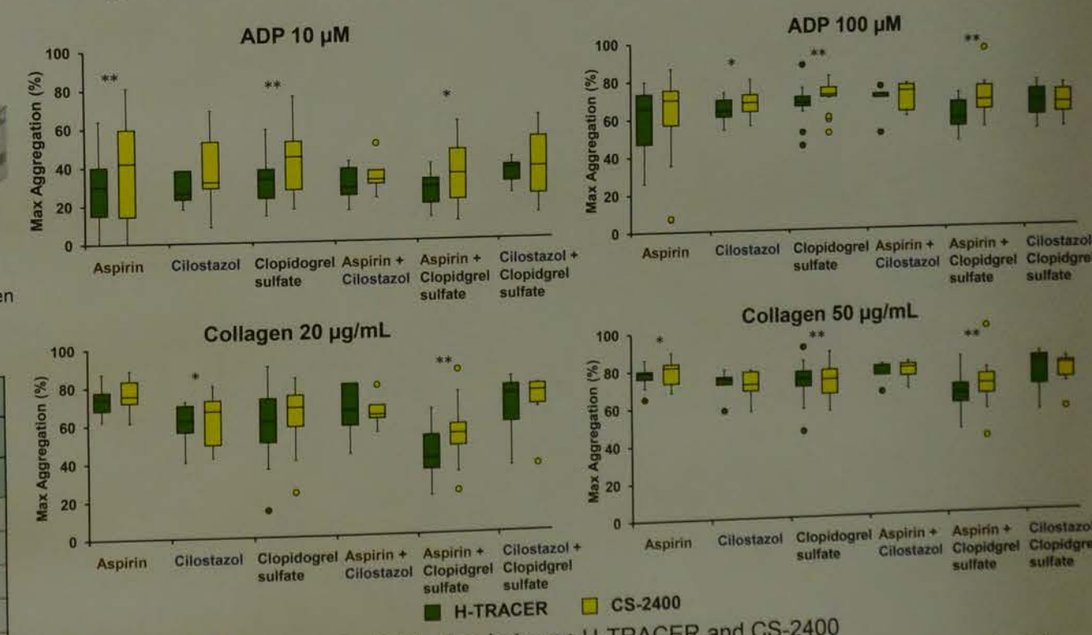


Figure 3 Some differences in distribution between H-TRACER and CS-2400
Results of the maximum aggregation in H-TRACER and CS-2400 were classified by the antiplatelet agents and shown in distribution plot. Some differences in distribution between two instruments was observed significantly, depending on the antiplatelet agent or its agonist. (Wilcoxon rank sum test ＊: $p < 0.05$, ＊＊: $p < 0.01$)

Discussion

Platelet aggregation test is useful for the diagnosis of platelet function and the assessment of antiplatelet drugs which occupied most of aim of this test in our hospital.

Our current study showed that within-run precision in CS-2400 and the correlation with the reference method were satisfactory and that interference agents were not significant effect on the platelet aggregation except it was necessary to pay attention to the hemolysis of samples.

The result of distribution plot of the maximum aggregation classified by the antiplatelet agents in H-TRACER and CS-2400 showed some significant differences between these instruments depending on each antiplatelet agent. It was suggested that the differences among lots of agonists and in the characteristics of the agonist by each manufacturer affected the aggregation curve and the maximum aggregation, while it seemed that less sample number was also one of the factor of it. So we need to perform this test upon understanding properly these characteristics of agonists or antiplatelet agents.

Conclusion

Our present study showed that the ability of platelet aggregation measurement on CS-2400 was satisfactory. Therefore, it was suggested that this automated technique would contribute to the standardization of platelet aggregation test.

C-53 C-54

Hematology

PC-54

Evaluation of the coagulation factor VIII and IX activity measurements using the chromogenic reagents

Madoka Inoue, Reiko Shizuka, Ayako Sato, Masaki Hyakawa, Katsuhiko Tsunekawa, Tetsuo Machida, Hiromi Koiso, Masami Murakami

Clinical laboratory center, Gunma University Hospital

【Introduction】

The use of the extended half-life (EHL) recombinant coagulation Factor VIII (FVIII) and Factor IX (FIX) was approved in Japan. The various EHL recombinant FVIII and FIX are developed, and it is hoped that these are approved in the near future in Japan. The coagulation factor activity of patients treated with some drug is reported to show different results between one-stage clotting assay and the chromogenic assay. We have evaluated the performance of coagulation FVIII and FIX using the chromogenic assay reagents.

【Materials and Methods】

We evaluated 1) Repeatability, 2) Reproducibility, 3) Linearity, 4) Limit of detection and 5) Interference testing. The activities of FVIII and FIX in three kinds of commercial plasma (Standard Human Plasma; SHP, control plasmas N; N and control plasmas P; P) were measured using new FVIII chromogenic reagent and new FIX chromogenic reagent on the automated coagulation analyser CS-5100 (Sysmex).

【Results】

1) Repeatability

We consecutively measured ten times of each N and P diluted to three densities. As for the coefficient variation (CV), FVIII was 0.3-3.8%, FIX was 0.6-1.1% (Table 1).

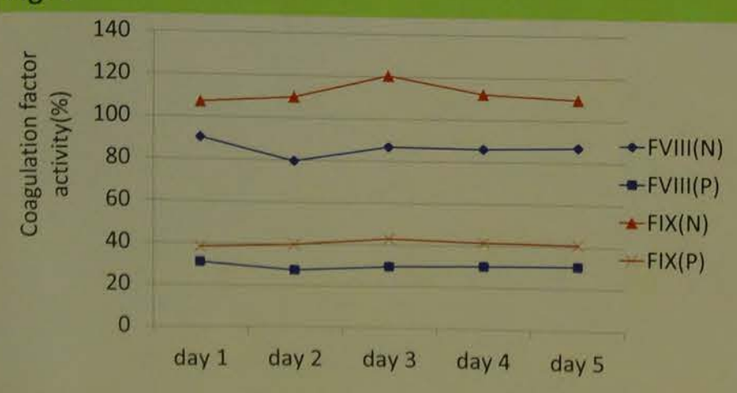
Table 1

	FVIII(%)				FIX(%)			
	N	P	1/4 P	1/8 P	N	P	1/4 P	1/8 P
1	79.9	27.7	6.9	3.6	109.8	40.1	9.1	4.7
2	79.5	27.6	6.7	3.4	110.0	39.7	8.9	4.7
3	79.6	27.5	6.6	3.3	109.5	40.0	8.8	4.6
4	79.3	27.3	6.6	3.3	109.9	40.1	8.9	4.7
5	79.3	27.4	6.5	3.3	108.4	39.9	9.1	4.6
6	79.9	27.7	6.8	3.5	108.0	39.9	9.0	4.6
7	79.9	27.7	6.6	3.4	110.5	40.2	8.9	4.7
8	79.8	27.4	6.7	3.3	109.4	40.2	9.1	4.6
9	80.1	27.3	6.6	3.2	109.7	40.5	9.0	4.7
10	79.8	27.1	6.4	3.2	110.0	40.4	8.9	4.6
CV(%)	0.3	0.7	2.2	3.8	0.7	0.6	1.2	1.1

2) Reproducibility

We measured N and P for 5 days (Fig. 1). Both FVIII and FIX CV were less than 5.0%.

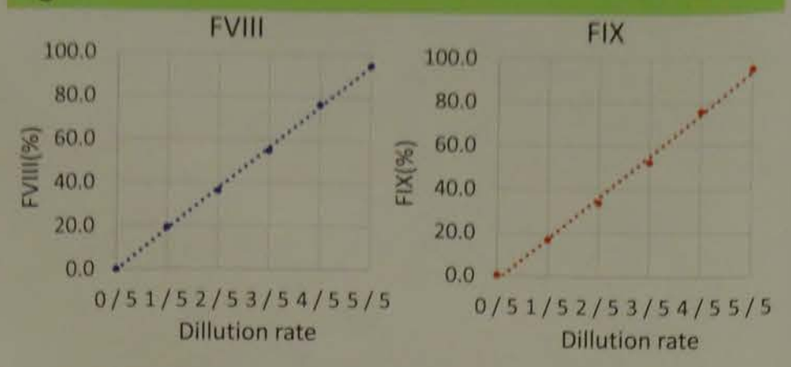
Fig. 1



3) Linearity

We measured SHP diluted to five densities using FVIII or FIX deficiency plasma. Good linearity was observed in both FVIII and FIX between around 100% and 0%.(Fig. 2).

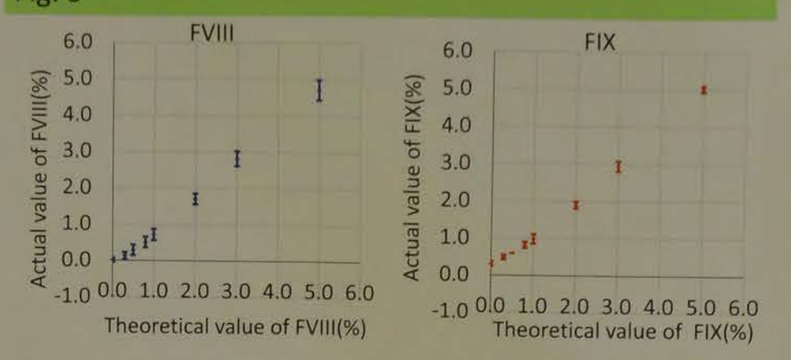
Fig. 2



4) Limit of detection

We measured each samples diluted SHP with saline ten times consecutively. We defined the limit of detection by 2SD method. As a result, FVIII was 0.5%, FIX was 0.3% (Fig. 3).

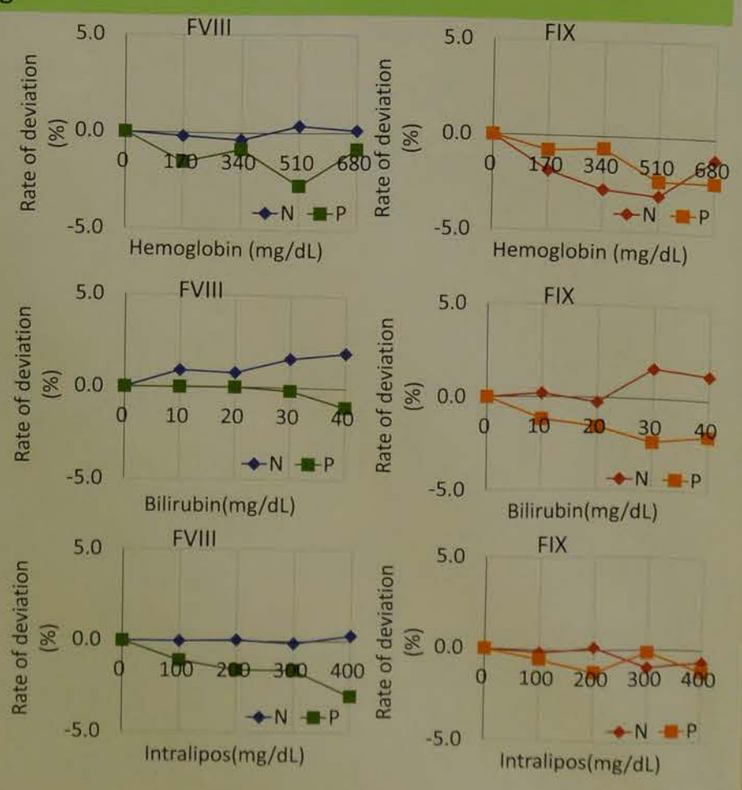
Fig. 3



5) Interference testing

We measured N and P spiked with hemoglobin, bilirubin and intralipos individually. Both FVIII and FIX were hardly affected by 680mg/dL hemoglobin, 40mg/dL bilirubin and 400mg/dL Intralipos (Fig. 4).

Fig. 4



【Conclusion】

Each basic examination about the performance of the FVIII and FIX activity measurements based on chromogenic assay showed good results, and both reagents will be useful in routine laboratory factor assay.

Usefulness of RET channel in XN-S

RBC-O, another RBC count parameter, by for blood specimens with cold hemagglu

Naomichi Tsuchiya, Kimiko Hioki, Katsu Yukinari Okayama, Fumihiko Nakamura

Tenri Yorozusoudansho Hospital

Introduction

omated hematology analyzers
red blood cell (RBC)-measuring
impedance-based RBC/platelet
to examine RBC count (RBC-I)
low cytometric reticulocyte (RET)
ed for reticulocyte measurements
gh the RBC/PLT channel provides
nts in most cases, analyzers show
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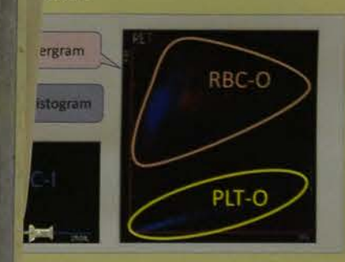

nce-based RBC-I and flow cytometric RBC-O

terials and Methods

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56 blood specimens with no
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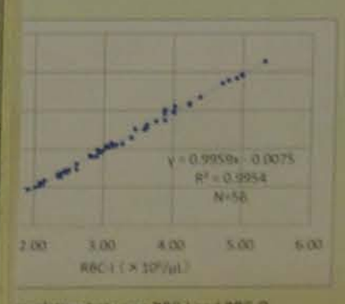
oglobin concentration (MCHC)
red in four specimens with cold
between two conditions: at room
or immediately after warming at
℃.

Results

elation ($y = 0.9959x - 0.0075$, $R^2 =$
:0.15 × 10⁶/μL was demonstrated
nd RBC-O in normal specimens



orrelation between RBC-I and RBC-O

Hematology

PC-55

Prediction of Myeloid Engraftment after Hematopoietic Stem Cell Transplantation Using an ADVIA2120i-derived parameter, BP ratio

Akishige Ikegame[1], Yusuke Inoue[1], Hiroshi Kanamori[2], Chihiro Inoue[1], Satiko Ogasa[1], Takayuki Nakao[1], Toshio Doi[3]
1) Division of Medical Technology, Tokushima University Hospital
2) Pathological department, Tokushima University Hospital
3)Division of Clinical Laboratory, Tokushima University Hospital

Purpose

The aim of this study is 1) to establish a new parameter, BP ratio, defined as %Blast suspects divided by %PMN, both of which are automatically calculated by a blood cell analyzer, ADVIA 2120i for predicting engraftment after hematopoietic stem cell transplantation (HSCT), and 2) to elucidate the relationship of BP ratio and morphological characteristics for better understanding of the cellular mechanism.

Methods

Blood samples of 34 engrafted and one rejected patients after HSCT were analyzed using ADVIA2120i. BP ratio was monitored along with other clinical variates. The cut-off value of BP ratio was determined using ROC curve analysis.
Morphological and surface marker-oriented characterization was performed using a May Giemsa and esterase staining on the cyto-spin samples, and flow cytometric analysis on CD45 gating.

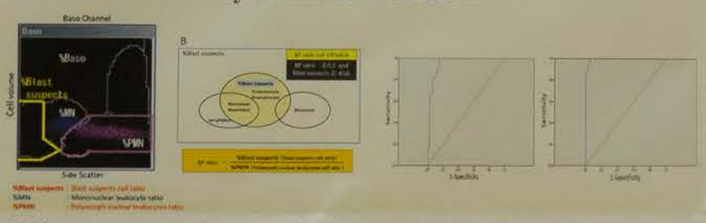
Background

Primary graft rejection, defined that WBC never increase more than 500/mL, occurs in 5-10% of patients undergoing allogeneic hematopoietic stem cell transplantation (HSCT), and results in a fatal clinical course unless a rescue HSCT is performed. Early and objective predictive methods of engraftment are desired. A new paremeter, BP ratio, is automatically calculated by a blood cell analyzer, ADVIA 2120i, and can be a promising predictor of the engraftment after HSCT, but the its relation to the cell population remains to be elucidated.

Results and Discussion

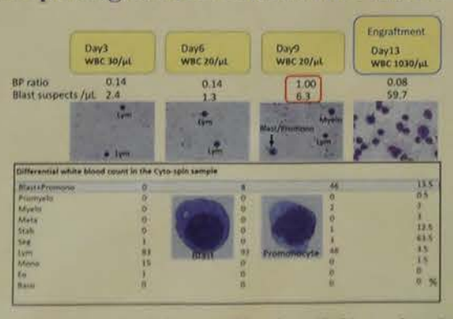
The cut-off value of BP ratio for predicting the engraftment was determined as 0.2.
The kinetics of BP ratio correlated with the kinetics of $CD45^{dim}$ $CD11b^-$ $CD36^-$ $HLA\text{-}DR^+$ blasts. After the peak of BP ratio, $CD15^+$ myeloid progenitors and $CD36^+$ promonocytes increased. These results suggests that BP ratio reflects the cell fraction of $CD45^{dim}$ $CD11b^-$ $CD36^-$ $HLA\text{-}DR^+$ blasts, and the transient increase of this cell population in early phase may be related to the engraftment.

1) Baso channel analysis in ADVIA2120i



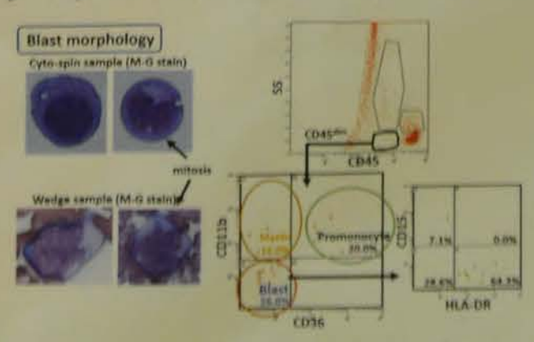
%Blast suspects are known to include myeloblasts, monoblasts, promyelocytes, promonocytes, and a small fraction of lymphoblasts and monocytes.
BP ratio was defined as %Blast suspects divided by %PMN.
ROC curve analysis revealed that BP ratio more than 0.2 is the most significant cut-off value.
To rule out the false positive due to low cell counts, Blast suspects > 4/mL was defined as a prerequisite.

2) Morphological cell counts after HSCT



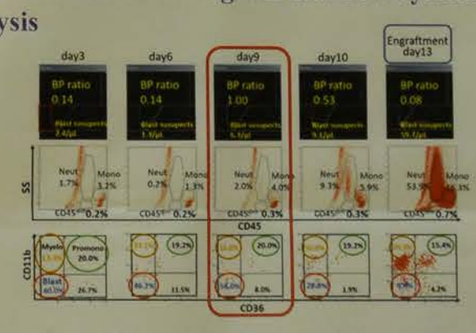
BP ratio exceeded the ct-off value (0.2) on day 6, and reached a peak on day 9 after HSCT. Cyto-spin samples were useful for differentiating the cells with low cell counts.

3) Morphological characterization of immature cells at the peak point of BP ration on day 9 after HSCT



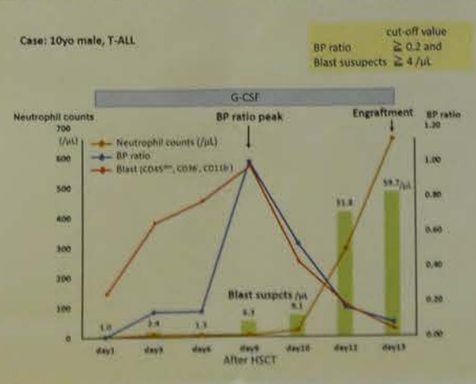
Morphological characterization of immature cells seen on day 9 revealed a number of myeloblasts and promonocytes with mitosis.

4) ADVIA 2120i scattergram and flow cytomerical analysis



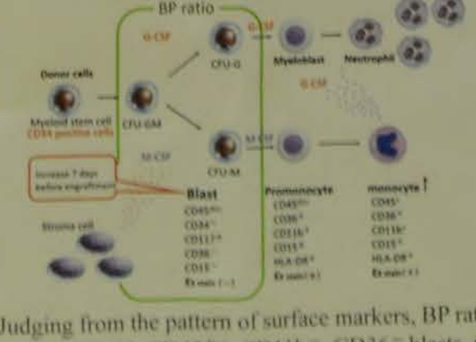
$CD45^{dim}$, $CD11b^-$, $CD36^-$ blasts were increased until day 9, and decreased subsequently.

5) Clinical course in a case of HSCT for T-ALL



$CD45^{dim}$, $CD11b^-$, $CD36^-$ blasts and BP ratio showed a similar kinetics.

6) Correspondence of BP ratio with cell fractions in the scheme of myeloid cell differentiation series



Judging from the pattern of surface markers, BP ratio correlated with $CD45^{dim}$, $CD11b^-$, $CD36^-$ blasts, which correspond with CFU-GM, CFU-G, and CFU-M.

Hematology

PC-56

Naomichi Tsuchiya, Kimiko Hioki, Katsuyo Tsuda, Yukinari Okayama, Fumihiko Nakamura
Tenri Yorozusoudansho Hospital

Introduction

The XN-Series automated hematology analyzers (Sysmex) have two red blood cell (RBC)-measuring channels; one is an impedance-based RBC/platelet (PLT) channel used to examine RBC count (RBC-I) and the other is a flow cytometric reticulocyte (RET) channel mainly used for reticulocyte measurements (Figure 1).Although the RBC/PLT channel provides accurate RBC counts in most cases, analyzers show false low RBC counts when analyzing agglutinated blood. Samples are immediately re-examined after warming. However, another RBC count parameter, RBC-O, is measured using the RET channel and can be used as a reference RBC count for specimens with cold hemagglutination.

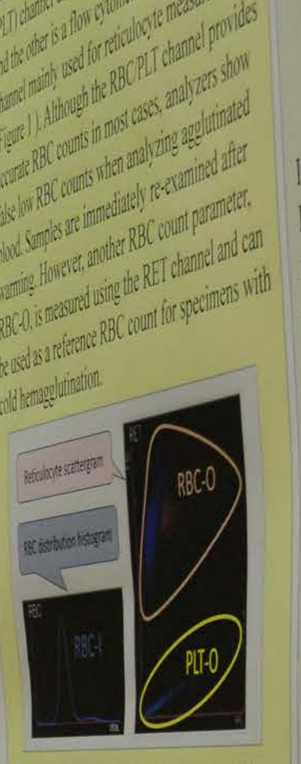


Figure 1. Impedance-based RBC-I and flow cytometric RBC-O

Materials and Methods

The correlation between RBC-I and RBC-O was examined using 56 blood specimens with no agglutination (normal specimens). These parameters and mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were then compared in four specimens with cold hemagglutination between two conditions: at room temperature (RT) or immediately after warming at approximately 40°C.

Results

An excellent correlation ($y = 0.9959x - 0.0075$, $R^2 = 0.9954$) within $\pm 0.15 \times 10^6$/μL was demonstrated between RBC-I and RBC-O in normal specimens (Figure 2-1,2-2).

Figure 2-2. Correlation between RBC-I and RBC-O

In the four spec
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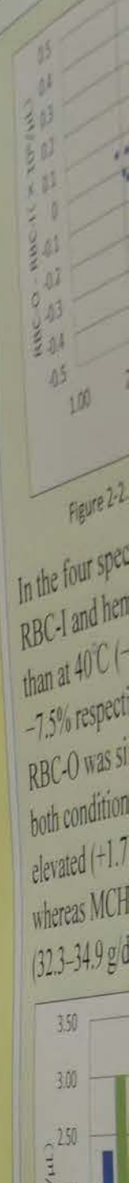

Figure 3. Cor
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Table 1. Com
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RBC-I (×10⁶/μL)
RBC-O (×10⁶/μL)
HGB (g/dL)
HCT (%)
MCV (fL)
MCH (pg)
MCHC (g/dL)

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C-3

Hematology

PC-56

Usefulness of RET channel in XN-Series automated hematology analyzers

RBC-O, another RBC count parameter, by RET channel provides accurate RBC count for blood specimens with cold hemagglutination

Naomichi Tsuchiya, Kimiko Hioki, Katsuyo Tsuda, Masashi Shimada, Yukinari Okayama, Fumihiko Nakamura

Tenri Yorozusoudansho Hospital

Introduction

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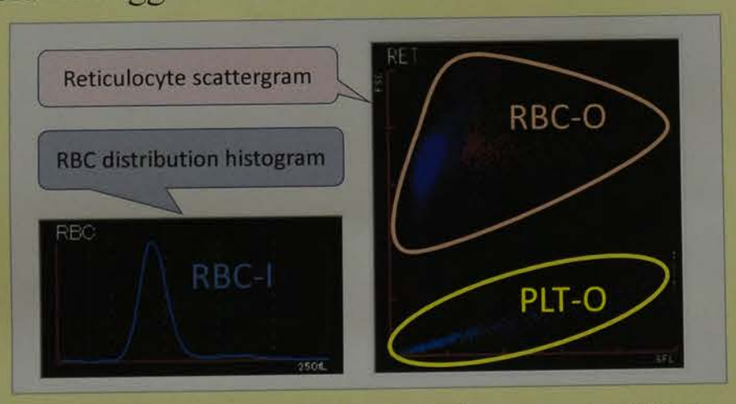

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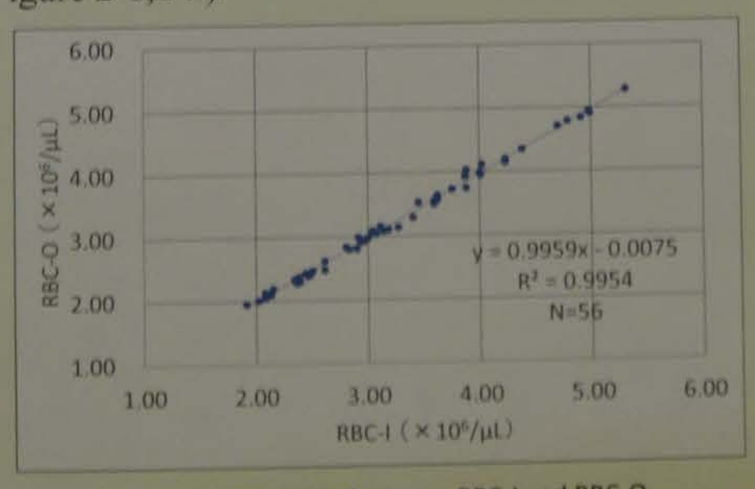


Figure 2-1. Correlation between RBC-I and RBC-O

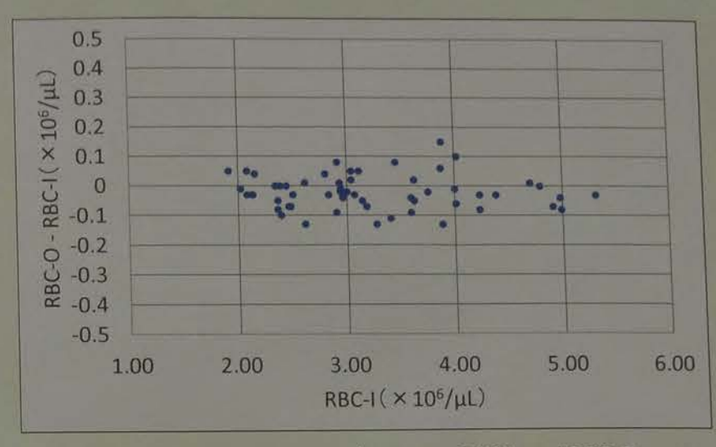


Figure 2-2. Discrepancy between RBC-I and RBC-O

In the four specimens with cold hemagglutination, RBC-I and hematocrit were significantly lower at RT than at 40℃ (−0.49 to −0.91 × $10^6/\mu L$ and −4.2 to −7.5% respectively)(Figure 3). On the other hand, RBC-O was similar (−0.04 to +0.15 × $10^6/\mu L$) under both conditions. MCV and MCHC were falsely elevated (+1.7 to +9.0 fL and 37.0–55.1 g/dL) at RT, whereas MCHC recovered to within normal range (32.3–34.9 g/dL) after warming (Table 1) .

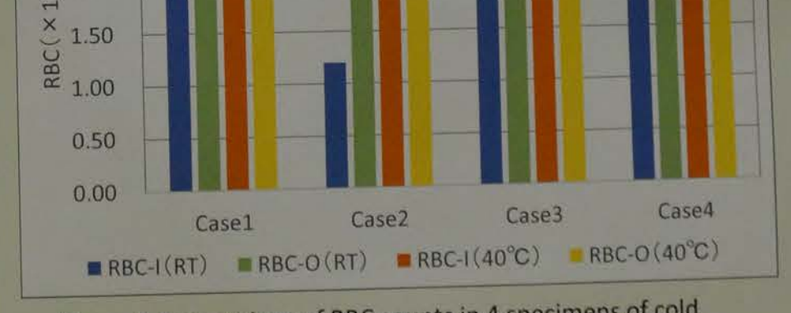


Figure 3. Comparison of RBC counts in 4 specimens of cold hemagglutination

Table 1. Comparison of RBC parameters and RBC indices under two conditions in 4 specimens of cold hemagglutination

	Case1		Case2		Case3		Case4	
	RT	40℃	RT	40℃	RT	40℃	RT	40℃
RBC-I(×10⁶/μL)	2.37	2.86	1.20	1.94	2.69	3.22	2.25	3.16
RBC-O(×10⁶/μL)	3.01	2.89	1.91	1.90	3.32	3.21	3.19	3.16
HGB(g/dL)	10.3	10.3	7.0	6.8	10.8	10.8	10.4	10.2
HCT(%)	25.1	29.5	12.7	20.2	29.2	33.4	24.1	31.0
MCV(fL)	105.9	103.1	105.8	104.1	108.6	103.7	107.1	98.1
MCH(pg)	43.5	36.0	58.3	35.1	40.1	33.5	46.2	32.3
MCHC(g/dL)	41.0	34.9	55.1	33.7	37.0	32.3	43.2	32.9

False low , False high

Discussion

The robustness (accuracy) of RBC-O may be because of the reaction temperature, 41℃, in the RET channel. Therefore, if RBC-I is significantly lower than RBC-O at RT, the specimen will have cold hemagglutination. If there is no discrepancy between RBC-I and RBC-O after warming, the specimen will not be affected by cold hemagglutination.

Hematology

PC-57

A case of TTP

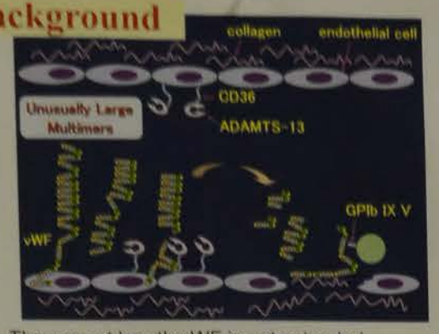
ELISA assay of ADAMTS13 and inhibitor were useful for the early treatment

○Toshiyuki Niiya[1)], Tatsuya Nishimiya[1)], Kazushi Tanimoto[2)], Taichi Azuma[2)], Takaaki Hato[2)]

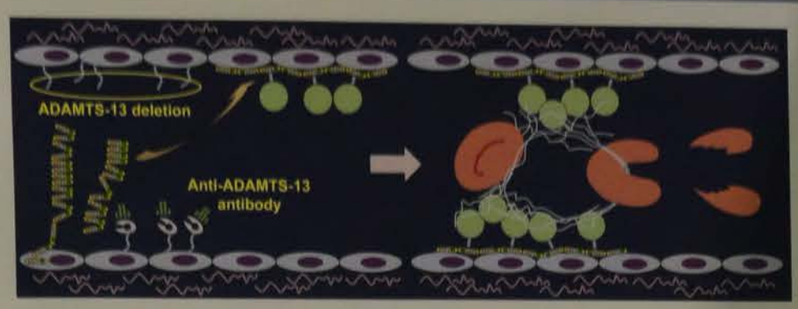
[1)] Department of Laboratory Medicine, Ehime University Hospital, Toon, Ehime, Japan

[2)] The first department of Internal Medicine, Ehime University Hospital, Toon, Ehime, Japan

Background



The correct length vWF is not extended with blood flow. If the vessels were damaged, these vWF can bind with endothelial collagen, and vWF will be extended. Then, hidden epitopes of vWF will appear, and the platelets bind to vWF by GPIbIXV expressed on the cell surface. Then activated platelets will gather and repair the injured tissue.

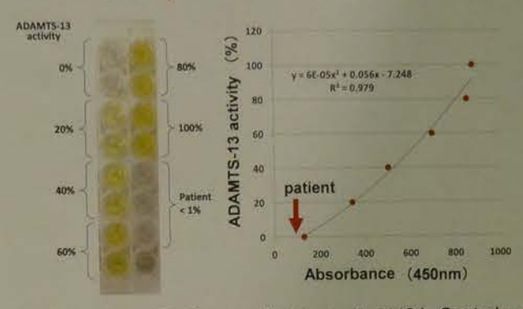


The patient with congenital defects of ADAMTS13 or acquired antibodies against ADAMTS13 cases, uncut ULM (Unusually Large Multimers) surrounds the systemic vessels.
In these situations, ULM is captured by very small vessels.
Extended vWF will attach at platelets and be activated on the surface of the platelet. After this, coagulant factors are activated and fibrin is formed.
But these fibrin nets caused fragmentation of red blood cells, as Schizocytes.
After these processes, Moschcowitz's five famous symptoms of TTP (Thrombocytopenia, Hemolytic anemia, fever, renal failure and neuropsychiatric symptom) will be seen.

Patient Case Study

- Case; 80s-year-old male, medicated for hypertension.
- Chief Complaint; Chest discomfort, loss of appetite.
- Labo. data; WBC 11.6 × 10^9/L, Hb 87g/L, PLT 7.0 × 10^9/L.
- Physical Examination.; Mild disturbance of consciousness, communication possible, free from dysarthria, no oral hemorrhage, no oral mucosa and scattering petechiae in the thoracicoabdominal part and the limbs.
- Head CT; Mild brain atrophy, no intracranial hemorrhage and acute infarction
- Past History; Prostate cancer resection at 60s y.o., appendicitis.
- Familial History.; N/A

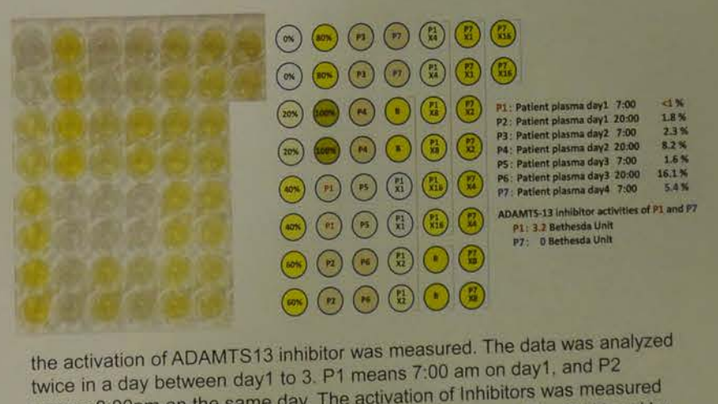
Analysis of ADAMTS13



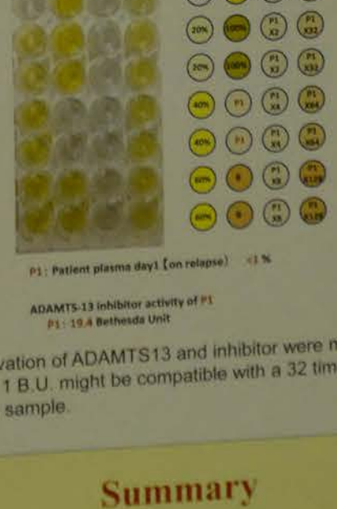
Activation of ADAMTS13 was calculated with ELISA. Controls were measured twice and patient samples were measured three times. We found the activation of ADAMTS 13 was less than 1%.

Laboratory data

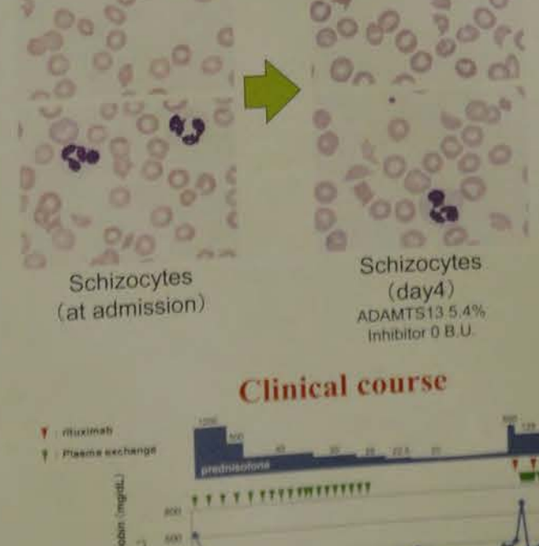
RBC	2.81 × 10^{12}	/L	TP	8.3	g/dL
Hb	83	g/L	Alb	4.3	g/dL
Ht	25.0	%	T.B	2.4	mg/dL
MCV	89.0	fL	AST	36	U/L
MCH	29.5	pg	ALT	15	U/L
MCHC	33.2	%	LD	615	U/L
			ALP	193	U/L
WBC	11.7 × 10^9	/L	BUN	32	mg/dL
Stab	1.8	%	Cr	1.33	mg/dL
Seg	74.6	%	Na	142	mmol/L
Ly	17.0	%	K	4.6	mmol/L
Mo	4.6	%	Cl	105	mmol/L
Eo	0.6	%	Fe	65	μg/dL
Ba	0.4	%	UIBC	274.0	μg/dL
At-Lym	0.4	%	Ferritin	659	ng/mL
Met	0.2	%	Haptoglobin	< 9	mg/dL
Myelo	0.4	%	CRP	0.56	mg/dL
NRBC	0.0	/100WBC			
Anisocyte	1+		APTT	26.5	sec
Poikilocyte	1+		PT	85.8	%
Schizocyte	+ (0.99%)		PT(INR)	1.05	
			Hepapl. T	121.6	%
PLT	7.0 × 10^9	/L	Fib	521	mg/dL
Reti	7.2	%	ATIII	125.7	%
			FDP	6.1	μg/mL
			FDP D-dimer	2.1	μg/mL



the activation of ADAMTS13 inhibitor was measured. The data was analyzed twice in a day between day1 to 3. P1 means 7:00 am on day1, and P2 means 8:00pm on the same day. The activation of Inhibitors was measured until day7. In this examination, P1 showed 3.2 Bethesda Units (B.U.) and by day7, after treatment, it came down to 0 B.U.



The activation of ADAMTS13 and inhibitor were measured on day230. 1 B.U. might be compatible with a 32 times dilution of patient's sample.



Schizocytes were detected until day40.

Clinical course

Thrombocytepenia and schizocytes were also appeared after treatment with plasma exchange and PSL. Laboratory data was improved except of schizocytes which were detected until day40.
This patient relapsed on day230. At that time, ADAMTS13 activity was less than 1% and Inhibitor was 19.4 B.U.
Patient was treated with PSL, plasma exchange plus CD20 antibody, "Rituximab" after relapse.

Summary

- This case is a representative case of TTP.
- ELISA assay of ADAMTS13 and inhibitor were useful for the early treatment of TTP
- PSL and plasma exchange(PE) was effective for first treatment, but Schizocytes were shown until day40.
- In the relapse phase, the activation of ADAMTS13 Inhibitor was higher than at diagnosis.
- PSL, PE and Retuximab treatment appear to be effective for this patient, so far.

COI

There are no relevant conflicts of interest to disclose

Hematology

PC-58

A rare case of acquired von Willebrand syndrome associated with systemic lupus erythematosus

Taku Nonaka, Michiko Yamazaki, Emi Suzuki, Naoko Maruyama, Yumiko Sakai, Ayano Yoshihara, Yuuki Tanaka, Takashi Yamada

Department of Medical Technology, Nagaoka Red Cross Hospital, Japan

1. Introduction

Acquired von Willebrand syndrome (AVWS) is a rare bleeding disorder that occurs in association with various underlying diseases. Approximately 700 AVWS cases have been reported, and systemic lupus erythematosus (SLE) is an infrequent cause of AVWS. Here we report on a rare case that led to the diagnosis of AVWS with SLE based on the results of detailed examinations.

2. Case report

A 67-year-old woman with no family history of bleeding disorders was found to have progressive anemia and black stools when she came to our hospital for dialysis. She was referred to the Department of Gastroenterology because upper gastrointestinal bleeding was suspected. She underwent upper gastrointestinal endoscopy and emergency hemostasis (Fig 1). The next day, she underwent endoscopy again to confirm hemostasis and reassess the source of bleeding, which led to a hemorrhage from the upper pharynx that caused uncontrolled bleeding. She was eventually referred to the Department of Hematology due to this bleeding tendency. Initial laboratory examinations revealed a prolonged activated partial thromboplastin time (APTT), markedly decreased factor VIII coagulant activity (FVIII:C), and severely decreased von Willebrand factor ristocetin cofactor activity (VWF:RCo) (Table 1). An APTT cross-mixing test (CMT) was performed, but the coagulation factor inhibitor was negative (Fig 2).

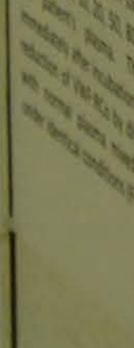


Fig 1. Endoscopic finding. Active bleeding near the duodenal papilla was observed.

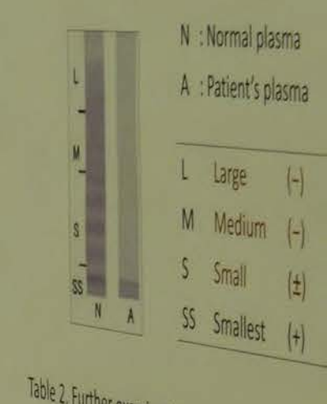
Table 1. Initial laboratory findings.

	Patient	Reference range
PT%	97%	79–107%
APTT	63.5s	28.8–40.2s
FVIII:C	6.2%	50–150%
FXI:C	105.6%	65–150%
VWF:RCo	<0.77%	70–150%

Fig 2. APTT CMT

3. VWF:RCo cross-mixing test

A CMT was performed by measuring VWF:RCo with a Von Willebrand factor (VWF) which contained stabilized platelets and normal plasma in six different mixtures of normal plasma and 0, 10, 20, 30, 80, and 100% concentrations of the patient's plasma. The mixtures were assayed immediately after incubation for 2 h at 37°C. To consider a reduction of VWF:RCo by dilution, a control was prepared with normal plasma mixed with saline and was tested under identical conditions (Fig 3).



4. Diagnosis

Fig 4. Multimeric analysis of VWF.

N : Normal plasma
A : Patient's plasma

L	Large	(-)
M	Medium	(-)
S	Small	(±)
SS	Smallest	(+)

Table 2. Further examinations.

	Patient	Reference ran
C3	55 mg/dL	65–135 mg/
C4	10 mg/dL	13–35 mg/d
ANA	1:40	<1:40
Anti-Sm Ab	78.1 U/mL	<9.9 U/mL
Lymphocyte	0.246 × 10^9 cells/L	>0.800 × 10^9
Anti-VWF Ab (ELISA)	IgG : 0.496	<0.100
	IgM : 0.089	<0.100

These findings (Fig 4 and Table 2) resulted in diagnosis of AVWS associated with SLE.

5. Discussion

If the presence of a coagulation factor inhibitor is su
APTT CMT is considered useful. In this case, we pe
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6. Conclusion

Although rare, if a patient with a bleeding te
encountered, we should always keep in mind the
of AVWS and perform detailed examinations
VWF:RCo CMT.

Hematology

PC-58

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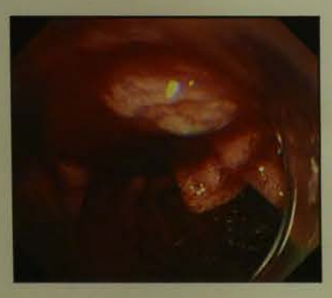

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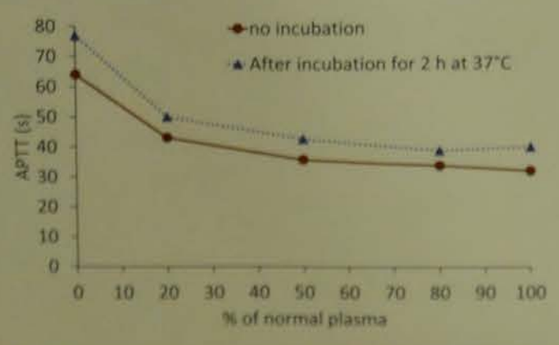

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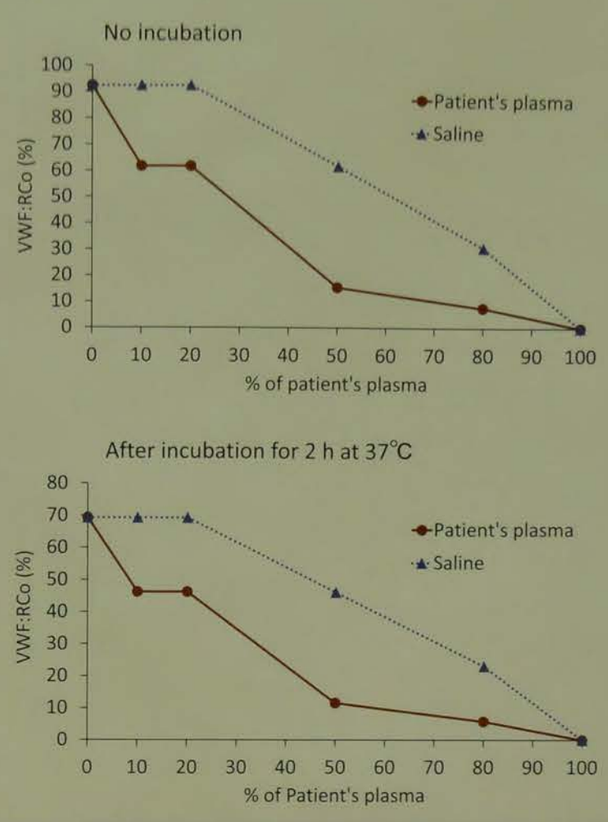
A CMT was performed by measuring VWF:RCo with a Von Willbrand reagent (Siemens), which contained stabilized platelets and ristocetin in six different mixtures of normal plasma and 0, 10, 20, 50, 80, and 100% concentrations of the patient's plasma. The mixtures were assayed immediately after incubation for 2 h at 37°C. To consider a reduction of VWF:RCo by dilution, a control was prepared with normal plasma mixed with saline and was tested under identical conditions (Fig 3).

Fig 3. VWF:RCo CMT.



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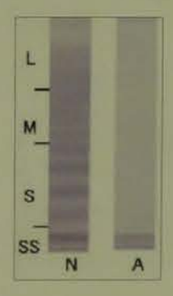

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These findings (Fig 4 and Table 2) resulted in the diagnosis of AVWS associated with SLE.

5. Discussion

If the presence of a coagulation factor inhibitor is suspected, APTT CMT is considered useful. In this case, we performed a VWF:RCo CMT as an additional test because the coagulation factor inhibitor was negative. As a result, the patient's plasma inhibited VWF:RCo in the presence of normal plasma, suggesting the presence of an inhibitor against VWF in the patient's plasma. Consequently, an IgG antibody against VWF was detected by enzyme linked immunosorbent assay (ELISA), and we concluded that VWF:RCo CMT is useful for the screening of a VWF inhibitor.

6. Conclusion

Although rare, if a patient with a bleeding tendency is encountered, we should always keep in mind the possibility of AVWS and perform detailed examinations such as VWF:RCo CMT.

Hematology

PC-59

A case report of an acquired factor V inhibitor.

Takeshi Osawa[1] Kazuo Kawasugi[2] Kimiko Nogi[1]
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1)Division of Laboratory Medicine, Teikyo University Hospital
2)Department of Internal Medicine, Teikyo University School of Medicine
3)Department of Laboratory Medicine, Teikyo University School of Medicine

1. Introduction

- The case of factor V inhibitor is rare. According to a systematic review[1], 159 reports of factor V inhibitor were emerged on literature. Among them, 78 reports were related to bovine thrombin usage, and others were related to non-bovine.
- We report a case of hemorrhagic tendency due to factor V inhibitor. And this case is negative about bovine thrombin usage.

2. History

- The patient is a 77years old man having been affected with cerebral infarction and hypertension. So he had taken clopidogrel, famotidine, perindopril, pitavastatin calcium, and amlodipine.
- In this time, due to coxalgia on his right side and swelling from subcutaneous hemorrhage, he became unable to walk. Therefore he was sent to our hospital.

3. Intervention/treatment

- On admission, laboratory findings went as Table1. In the first, the lack of vitamin K was suspected. But infusion of fresh-frozen plasma and administration of vitamin- K couldn't correct coagulopathy.
- After 4 days in our hospital, mixing tests suggested presence of an inhibitor(Figure 1-2). Assays for specific coagulation factors and factor-inhibitors were performed, which confirmed the presence of a factor V inhibitor(8.9BU).

Table 1 Laboratory Findings on admission

CBC		Coagulation		Biochemistry			
WBC	12.1 X10³/l	PT	56.2 sec	TP	6.5 g/dL	CK	1331 U/l
RBC	2.08 X10⁴/l	PT%	14.0 %	ALB	3.1 g/dL	UN	48.7 mg/dL
Hb	6.4 g/dL	PT-INR	4.08	T-Bil	0.54 mg/dL	creatinine	2.39 mg/dL
Ht	18.6 %	APTT	177.8 sec	AST	37 U/L	Na	128 mEq/l
MCV	89.4 fl	FIBG	457 mg/dL	ALT	20 U/L	K	5.2 mEq/l
MCH	30.8 %			LDH	209 U/L	Cl	90 mEq/l
MCHC	34.4 %			ALP	171 U/L	Ca	8.6 mEq/l
PLT	321 X10³/l			r-GTP	15 U/L	CRP	1.64 mg/dl
				AMY	163 U/L		

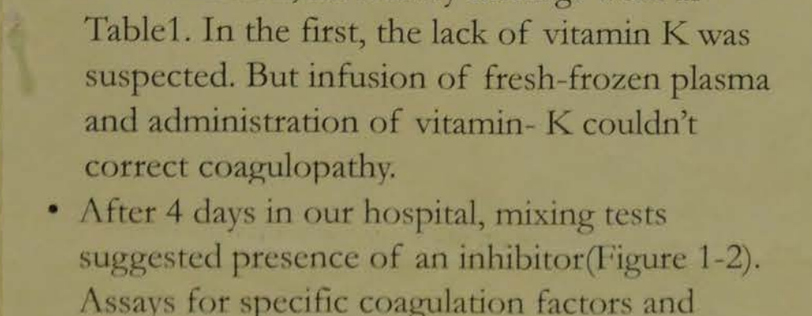


Fig.1 PT Mixing test (4th day after admission)
Fig.2 APTT Mixing test (4th day after admission)

- On 7th hospital day, oral prednisolon was administered. Then the coagulation parameters improved gradually. And factor V inhibitor decreased to 2.5BU on 21st hospital day(Figure 3).
- On 39th day, coagulation parameters fell within reference interval : PT 15.3 sec, PT-INR 1.16, APTT 29.2 sec, F.V 100%, F.V inhibitor 0.1> BU.

Table 2 Coagulation factors and inhibitors

	[%]		[BU]
F.Ⅱ	52	F.Ⅱ inhibitor	0.1>
F.Ⅴ	1	F.Ⅴ inhibitor	8.9
F.Ⅶ	48	F.Ⅶ inhibitor	0.1>
F.Ⅷ	126	F.Ⅷ inhibitor	0.1>
F.Ⅸ	110	F.Ⅸ inhibitor	No data
F.Ⅹ	60	F.Ⅹ inhibitor	0.1>

Table 3 Cause of F.V inhibitors

Condition associated with the development of F.V inhibitors	Frequency*	In this case
Antimicrobial drugs	42%	○
Surgical procedures	31%	×
Infection	23%	▲
Cancer	22%	×
No underlying causes	21%	–
Autoimmune disorders	13%	×
Transplants and blood transfusion	5%	▲
clopidogrel	?	○

*Frequency among non-bovine cases.
The number of bovine thrombin-associated is 78/159.[1]

Hospital days	4	17	24	31	38	45
F.V [%]	1	2	14	59	100	104
F.V inhibitor [BU]	8.9	7.7	2.5	1.6	0.1	0.1

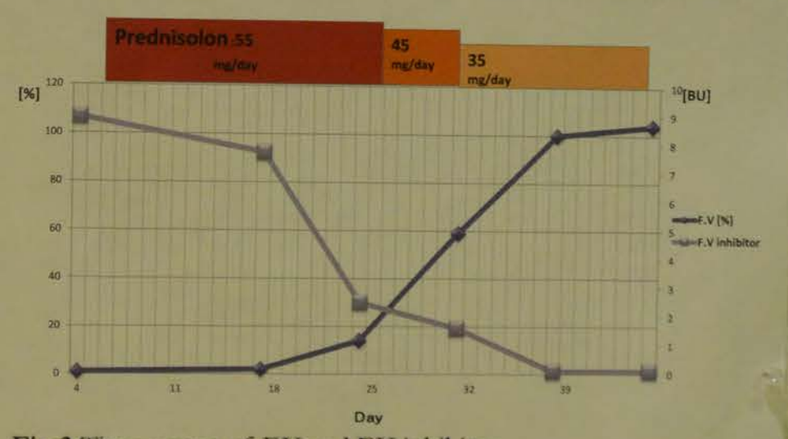


Fig.3 Time course of F.V and F.V inhibitor.

4. Discussion

- We performed a search on Pub Med using the term "acquired factor five inhibitor" without time limits. There are 170 reports about this term, and 21 of them are written by Japanese authors.
  The same search on Japan Medical Abstracts Society as Pub Med are performed. A hit number is 90. So it can be said that the case of factor five inhibitor is rare, and making a report of this case is significant .
- In recent years, many non-bovine related F.V inhibitor have reported. Table.3 shows conditions associated with the development of F.V inhibitor in these report.
- In this case, the patient had taken oral anti-platelet drug. To our knowledge, this is the first cace of F.V inhibitor in a patient taking anti-platelet drug(clopidogrel).

References

- 1)Franchini M,Lippi G. Acquired factor V inhibitors: a systematic review. J Thromb Thrombolysis. 2011;31:449-457
- 2)Collins PW, Hirsh S, Baglin TP, et al. Acquired hemophilia A in the United Kingdom: 3-year national surveillance study by the United Kingdom Haemophilia Centre Doctors' Organisation. Blood. 2007;109:1870-1877.

I have no COI
with regard to our presentation.

Hematology

PC-60

One case of the articular rheumatism that developed a hemophagocytic syndrome

Kazuhiro Ichishita, Tiduko Nonaka, Erina Sibata, Yasuyo Hirakawa, Kaoru Urakawa, Kazuma Taniguti, Yasusi Kawabuti, Katuyuki Nagatoya

Japan Organization of Occupational Health and Safety Osaka Rosai Hospital

Introduction

Hemophagocytic syndrome (HS) is occasionally associated with autoimmune diseases, such as juvenile rheumatoid arthritis (RA) and Still' s disease. However, HS related to adult onset RA has been rarely reported.

Case

A male patient in his 70s who had developed RA 4 years earlier underwent methotrexate therapy for RA deterioration in June . The initial dose was 6 mg/week, which was increased to 8 mg/week in July. He was diagnosed with pancytopenia in October. On admission to Osaka Rosai Hospital, his white blood cell (WBC) count was 0.5 × 10^9/l, red blood cell (RBC) count was 215 × 10^{12}/l, and platelet count was 46 × 10^9/l.

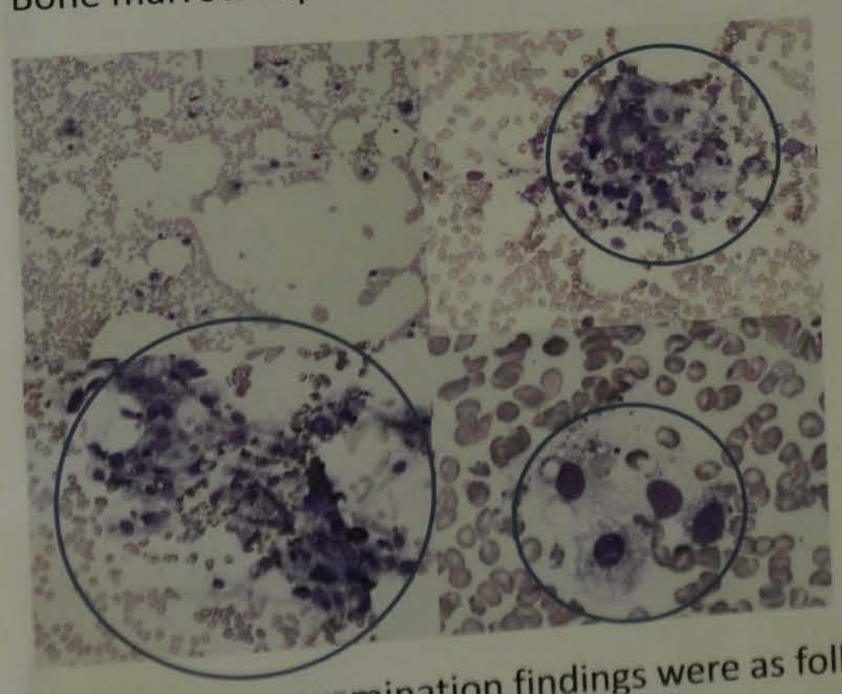
On admission blood test findings

WBC	0.5	10^9/l	Na	138	mEq/l	CPK	17	U/l
RBC	2.15	10^{12}/l	K	4.9	mEq/l	Glu	115	mg/dl
Hb	7.7	g/dl	Cl	110	mEq/l	BUN	27	mg/dl
Hct	22.1	%	Ca	9.0	mg/dl	Cre	1.4	mg/dl
PLT	46	10^9/l	TP	5.6	g/dl	UA	5.9	mg/dl
Neutro	88.6	%	Alb	2.7	g/dl	CRP	32.86	mg/dl
Lymph	8.8	%	T-Bil	1.1	mg/dl	CMV-IgG		(+)
Mono	2.6	%	AST	39	U/l	CMV-IgM		(−)
Eosin	0.0	%	ALT	26	U/l	EB VCA IgG		(+)
Baso	0.0	%	LDH	215	U/l	EB VCA IgM		(−)
ESR	154	mm/h	ALP	245	U/l	EB EBNA		(+)

Treatment

Because pancytopenia was assumed to be induced by methotrexate, it was discontinued, and folate and granulocyte colony-stimulating factor (G-CSF), antibiotics, and gamma globulin preparations were administered. The patient also underwent RBC and platelet transfusions. Nonetheless, the WBC count did not increase, and he developed thrombocytopenia. Bone marrow aspiration was performed on day 5. The total nucleated cell count decreased to 0.7 × 10^9/l, consisting 17.6% macrophages. Hemophagocytosis was diffusely observed using microscopy

Bone marrow aspiration



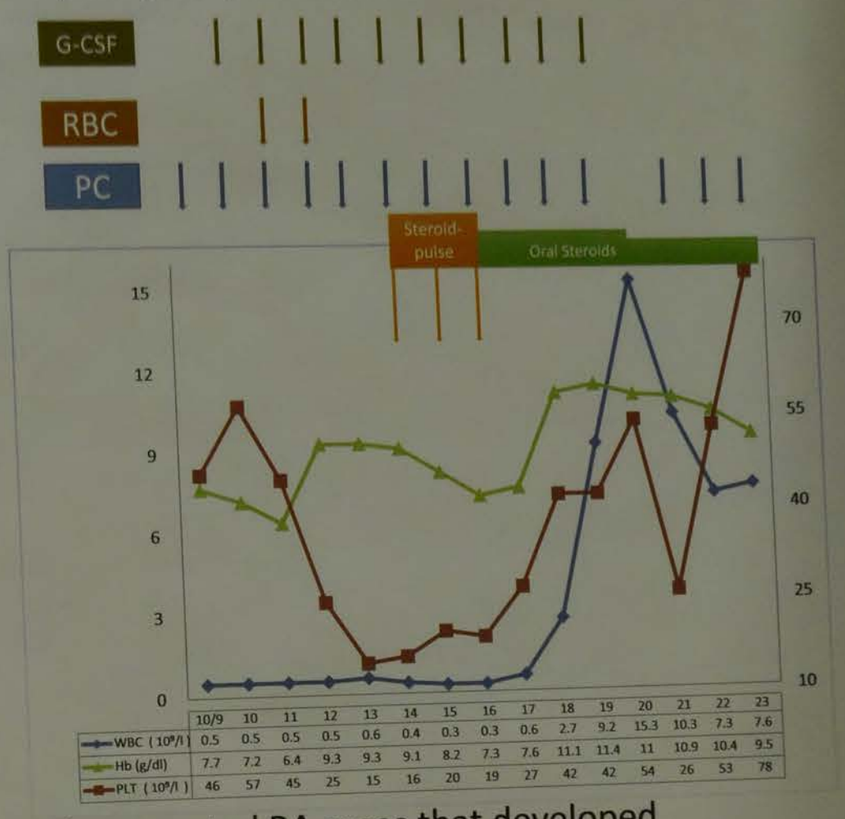
Bone marrow examination findings were as follows

NCC	7000	/μl	Mybl	0.0 %
MgK	6	/μl	Pro	0.6 %
M/E	4.8		Myelo	1.0 %
W/E	23.8		Meta	0.4 %
			Eosino	18.0 %
			Lympho	52.0 %
			Mono	2.0 %
			Plasma	2.8 %
			B Ebl	0.2 %
			P Ebl	2.0 %
			N Ebl	2.0 %
			Atypical lym	1.4 %
			Macrophage	17.6 %

Bone marrow hypoplasia was observed. The margins of the specimen had an increased number of macrophages, and phagocytosis was observed. There were no malformed cells in the blood specimen. Hemophagocytic syndrome was diagnosed.

Treatment (steroid pulse, G-CSF, transfusion)

His treatment involved steroid pulses, G-CSF, and transfusions. He underwent steroid pulse therapy for 3 days, after which the steroid levels were decreased. G-CSF administration and transfusion were performed over time. The patient gradually recovered.



	10/9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
WBC (10^9/l)	0.5	0.5	0.5	0.5	0.6	0.4	0.3	0.3	0.6	2.7	9.2	15.3	10.3	7.3	7.6
Hb (g/dl)	7.7	7.2	6.4	9.3	9.3	9.1	8.2	7.3	7.6	11.1	11.4	11	10.9	10.4	9.5
PLT (10^9/l)	46	57	45	25	15	16	20	19	27	42	42	54	26	53	78

The reported RA cases that developed hemophagocytic syndrome

Case	Age	Sex	RA history	Therapy for RA	Opportunistic infection	Therapy for HPS	Prognosis	Author	
1	61	F.	UNK.	PSL	Enterobacter sepsis	UNK.	recovered	Reiner	1988
2	63	F.	3y	UNK.	(-)	PSL 40mg daily	recovered	Onishi	1994
3	64	F.	4y	D-penicillamine, parenteral gold, CyA,MTX,anti-CD4 antibodies,MTX+ sulfasalazine+PSL	Escherichia coli septicemia	PSL 15mg daily+antibiotics	recovered	Sibilia	1998
4	62	M.	16y	MTX	(-)	PSL 40mg daily	recovered	Yamanouchi Jun	1998
5	61	F.	20y	operation,MTX+ PSL+auranofin	Small intestine perforation due to cytomegalovirus Infections	G-CSF,γ-globulin,mPSL-pulse ,ganciclovir,operation	recovered	Kuyama Jun	2000
6	63	M.	9y	DMARD,MTX, CY+PSL,operation	Cytomegalovirus Infections	mPSL-pulse,PSL 80-50mg daily	died	Kihara Toru	2002
7	58	M.	17y	MTX+CyA+DFC	visceral leishmaniasis	UNK.	recovered	Horcada Ml	2003
8	60	F.	3y	MTX,mPSL	(-)	PSL 40mg daily,IVCY+ γ-globulin+antibiotics	recovered	Naoshi Sakai	2003
9	70	M.	4y	MTX	(-)	mPSL-pulse +G-CSF+γ-globulin+ Transfusion+antibiotics	recovered	Present case	

drug:MTX,methotrexate;mPSL,methylprednisolone;PSL,prednisolone , IVCY,intravenous pulse cyclophosphamideUNK.,unknown

"Hemophagocytic syndrome associated with rheumatoid arthritis" some reorganization

Discussion

HS associated with viral infections can occur in adult RA patients. However, a viral infection appeared unlikely in this case. Methotrexate is frequently administered before the onset of HS coincident with RA, indicating that RA activity is exacerbated in these patients. In this case, HS appears to have been caused by severe RA or a viral infection (except Epstein-Barr virus or cytomegalovirus) during pancytopenia because of the methotrexate. Drug-induced pancytopenia associated with HS can become quite severe and even lethal if not diagnosed on time.

Conclusion

HS coincident with RA was evaluated. In patients with pancytopenia, bone marrow aspiration should be promptly considered for early HS diagnosis.

Hematology

PC-61

A case of sickle cell disease with abnormally high level of HbA1c

Hisami Baba[1], Shouhei [illegible]

1)Nagano Rederos

2)Nagano Rederos

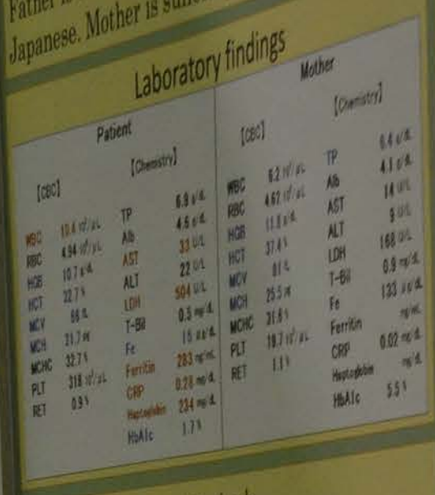
Introduction

We experienced a case of sickle cell disease with abnormally high level of HbA1c. Also conventional HPLC chromatograph showed the presence of abnormal peak but only targetcells in peripheral blood smear.We report the morphological change after 2 hours using a simple method in this case.

Patient

6 year old female with back pain and stomachache. Parvovirus B19 IgM antibody positive. Father is a half of Brazilian and African. Mother is Japanese. Mother is suffering from mild anemia.

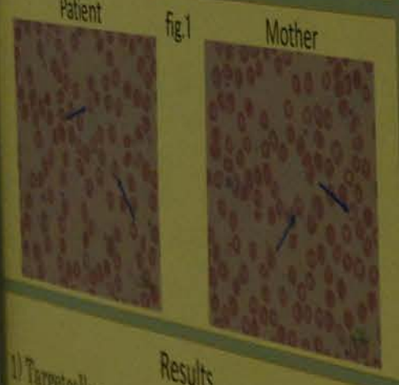
Laboratory findings



Methods

1) We observed peripheral blood smears of the patient and mother.
2) Their HbA1c were measured using HLC-723G9 and affinity mode of HLC-723G8.
3) Their hemoglobin were analysed using conventional HPLC and β- thalassemia mode of HLC-723G8 at Tosoh Corporation.
4) We put 0.5 drops of their blood on each slide glass and put cover glass on their blood to produce anaerobic condition,and we observed morphological change after 2 hours (sickle cell forming test).

Patient fig.1 Mother



Results

1) Targetcells were observed on their peripheral blood smears(fig.1).
2) HbA1c of the patient was 1.7%, which was considerably lower than the reference values (4.6 - 6.2%).HbA1c of her mother was 5.5%,which was in the reference value and the affinity mode showed that HbA1c 1.7% of the patient was false low level. Also,any abnormal peaks weren't detected in their G9 chromatograms(fig.2).
3) Conventional HPLC showed that the patient had a abnormal peak but her mother didn't have any abnormal peak(fig.3). In β-thalassemia mode, HbA2 and HbS of the patient were 5.6% (2.0 -3.5%) and 54.2% (0.0%). The other side,her mother's values were each 5.7% and 0.0% (fig.4).
4) The patient's erythrocytes changed their shape to sickle-like in anaerobic condition,but mother's didn't change their shape(fig.5).

Hematology

PC-61

A case of sicklecell disease with abnormally low level of HbA1c by the HPLC method

Hisami Baba[1)], Shouhei Nakata[2)], Takayoshi Tokutake[2)]

1)Nagano Redcross Hosp.Dept.of Clinical Labo.

2)Nagano Redcross Hosp.Dept.of Blood Transfusion

Introduction

We experienced a case of sickle cell disease with abnormally low level of HbA1c by the HPLC method. Also conventional HPLC chromatograph showed the presence of HbA2 and HbS in the patient,but we observed only targetcells in peripheral blood smear.We report the morphological change of the erythrocytes to sickle cells using a simple method in this case.

Patient

6 year old female with back pain and stomachache.
Parvovirus B19 IgM antibody positive.
Father is a half of Brazilian and African. Mother is Japanese. Mother is suffering from mild anemia.

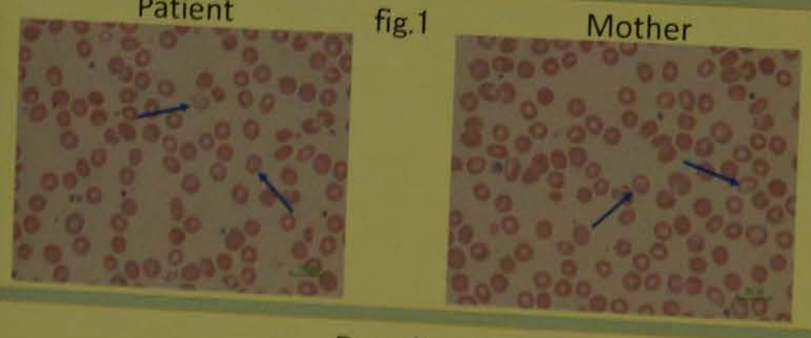
Laboratory findings

Patient				Mother			
【CBC】		【Chemistry】		【CBC】		【Chemistry】	
WBC	10.4 $10^3/\mu L$	TP	6.9 g/dL	WBC	6.2 $10^3/\mu L$	TP	6.4 g/dL
RBC	4.94 $10^6/\mu L$	Alb	4.6 g/dL	RBC	4.62 $10^6/\mu L$	Alb	4.1 g/dL
HGB	10.7 g/dL	AST	33 U/L	HGB	11.8 g/dL	AST	14 U/L
HCT	32.7 %	ALT	22 U/L	HCT	37.4 %	ALT	9 U/L
MCV	66 fL	LDH	504 U/L	MCV	81 fL	LDH	168 U/L
MCH	21.7 pg	T-Bil	0.5 mg/dL	MCH	25.5 pg	T-Bil	0.9 mg/dL
MCHC	32.7 %	Fe	15 μg/dL	MCHC	31.6 %	Fe	133 μg/dL
PLT	318 $10^3/\mu L$	Ferritin	283 ng/mL	PLT	19.7 $10^3/\mu L$	Ferritin	ng/mL
RET	0.9 %	CRP	0.28 mg/dL	RET	1.1 %	CRP	0.02 mg/dL
		Haptoglobin	234 mg/dL			Haptoglobin	mg/dL
		HbA1c	1.7 %			HbA1c	5.5 %

Methods

1) We observed peripheral blood smears of the patient and mother.
2) Their HbA1c were measured using HLC-723G9 and affinity mode of HLC-723G8.
3) Their hemoglobin were analysed using conventional HPLC and ß- thalassemia mode of HLC-723G8 at Tosoh Corporation.
4) We put 0.5 drops of their blood on each slide glass and put cover glass on their blood to produce anaerobic condition,and we observed morphological change after 2 hours (sickle cell forming test).

fig.1



Results

1) Targetcells were observed on their peripheral blood smears(fig.1).
2) HbA1c of the patient was 1.7%, which was considerably lower than the reference values (4.6 - 6.2%).HbA1c of her mother was 5.5%,which was in the reference value and the affinity mode showed that HbA1c 1.7% of the patient was false low level. Also,any abnormal peaks weren't detected in their G9 chromatograms(fig.2).
3) Conventional HPLC showed that the patient had a abnormal peak but her mother didn't have any abnormal peaks(fig.3). In ß-thalassemia mode, HbA2 and HbS of the patient were 5.6% (2.0 -3.5%) and 54.2% (0.0%). The other side,her mother's values were each 5.7% and 0.0% (fig.4).
4) The patient's erythrocytes changed their shape to sickle-like in anaerobic condition,but mother's didn't changed their shape(fig.5).

fig.2

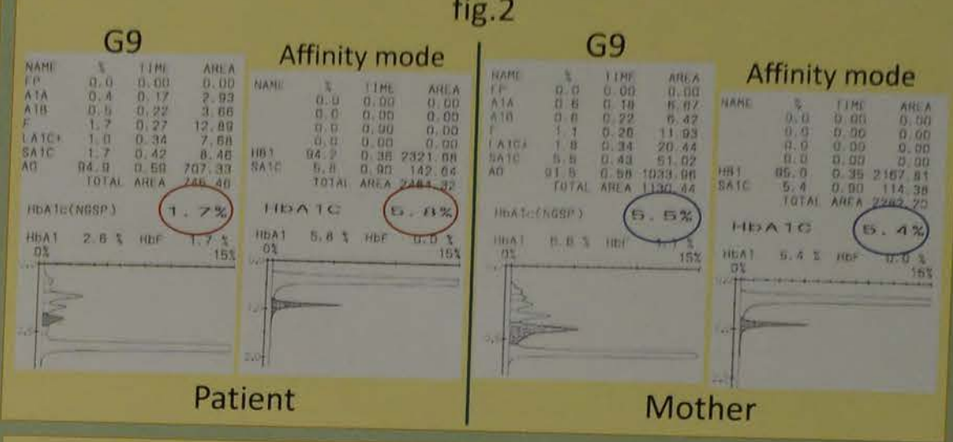


fig.3

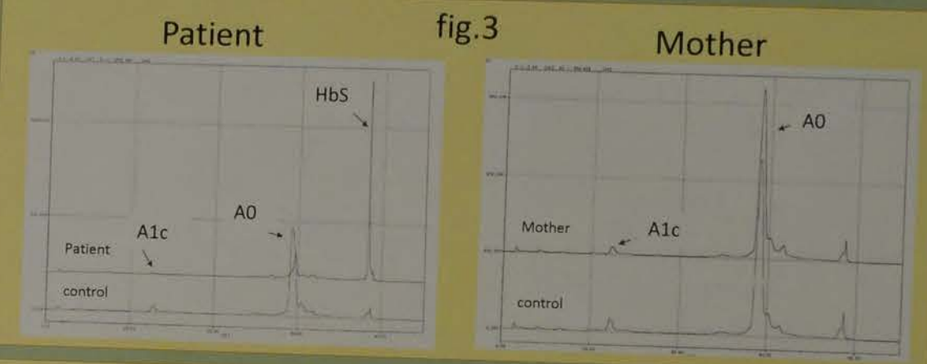


fig.4

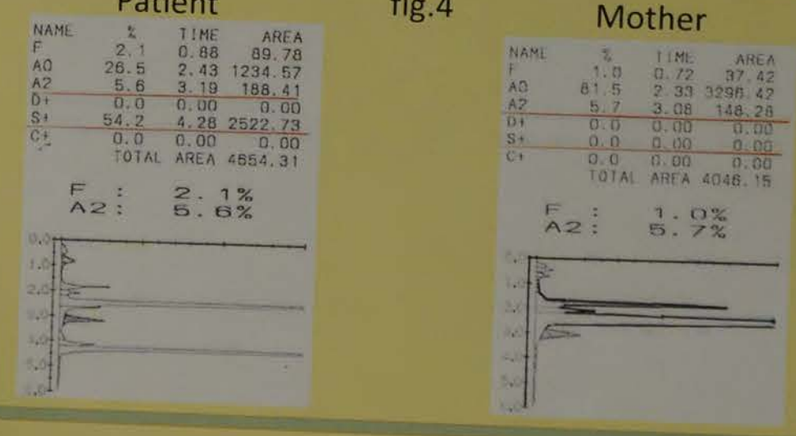
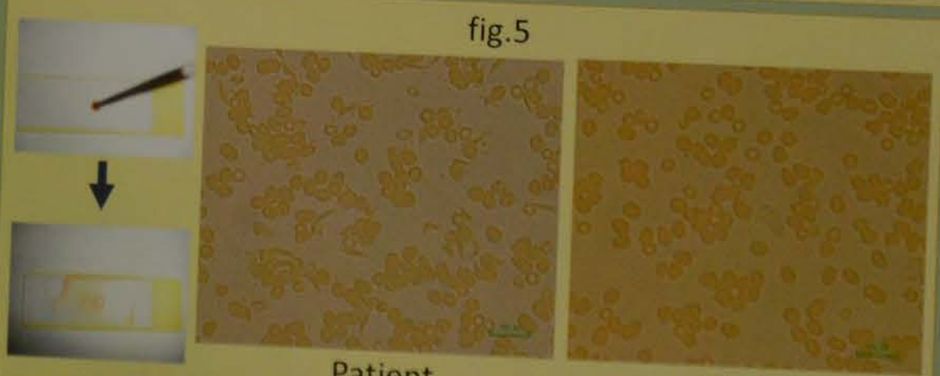


fig.5



Conclusion

Since targetcells were observed in peripheral blood of the patient and mother, we suspected that they were thalassemia,but hemoglobin analysis by the HPLC method showed that not only the patient had HbS but also the patient and mother had high level of HbA2.So the reason of abnormally low level of HbA1c in the patient was considered to be caused by high level of HbS. The patient was finally diagnosed [compound heterozygote of HbS and ß-thalassemia].In the case of heterozygosis HbS, erythrocytes change their shape to sickle-like only in anaerobic condition,So on this case,pain from vaso-occlusive episode and bone marrow necrosis triggered by parvovirus B19 infection were suspected. In this study,we succeeded in making sickle cell with simple method.Sickle cell forming test was useful for diagnosis of sickle cell disease.

Hematology

PC-62

Development of an internal quality control application for immature granulocyte differential

Naoya Ichimura, Ayako Itoi, Yuki Kouda, Yuki Okubo, Michio Hagihara, Syuji Tohda

Clinical Laboratory, Medical Hospital of Tokyo Medical and Dental University

Objectives

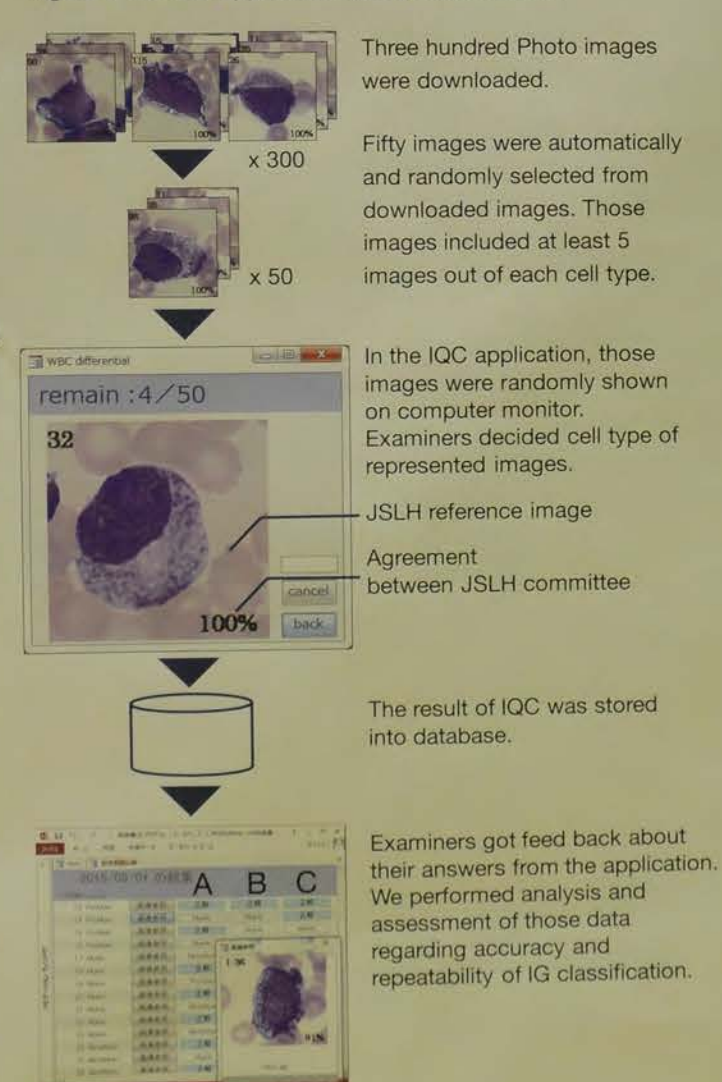
Thus far, there are no internal quality control (IQC) procedure for counting the percentage of immature granulocyte (IG) on blood smears. We developed an IQC application to assess the examiner's performance and to standardize criteria for IG classification. The aim of this work is to evaluate an inter-observer agreement of correctly recognizing IG.

Method

The IQC application for IG classification

The application was developed using Microsoft Access 2013. Three hundred photo images were downloaded from website of the Japanese Society for Laboratory Hematology (JSLH, http://www.jslh.com), opened to standardize the morphological counting of WBC differential. Those images included myeloblast, promyelocyte, myelocyte, metamyelocyte, atypical lymphocyte and normal lymphocyte (Figure 1).

Figure 1 The IQC application for IG classification



Analysis and assessment of IQC results

Three examiners performed IQC using this application for 70 days (Table 1). The following contents were compared between first half period (35 days) and second half period (35 days).

1. Agreement between examiner and JSLH (Cohen's κ coefficient)
2. The change of IG classification by each examiner
3. The change of agreement in each cell type

Table 1 Work history of examiners for WBC differential

Examiner	Work history
A	1 year 2 month
B	4 year 2 month
C	3 year 6 month

Result 1
Agreement between examiner and JSLH

In order to quantitatively evaluate agreement between examiner and JSLH, Cohen's κ coefficients were calculated in IG classification except for lymphocyte. These values in second half period got larger than those in first half period and were more than 0.9 in all examiners.

Table 2 Cohen's κ coefficients

Examiner	First	Second
A	0.80	0.94
B	0.85	0.96
C	0.93	0.99

First : First half period (35 days)
Second : Second half period (35 days)

Result 2
The change of IG classification by each examiner

The cell type which examiners determined reference images was analyzed. In myeloblast and metamyelocyte, agreements were extremely high both first and second half period. In promyelocyte and myelocyte, JSLH reference images were classified more than 5% into more mature or immature cell type, respectively, depending on examiners. But, those miss-classification were suppressed to less than 5 % in second half period except for myelocyte in examiner A.

Table 3 The breakdown of IG classification

First half period

Examiner		JSLH Blast	Pro	Myelo	Meta	Aty.Lym	Lym
A	Blast	99.1	0.5				0.5
	Pro		90.4	11.2			
	Myelo		9.1	82.8	3.9		
	Meta			6.0	96.1		0.5
	Aty.Lym	0.9				92.2	16.5
	Lym					7.8	82.5
B	Blast	97.8	0.5			1.1	2.0
	Pro		85.2	1.7			
	Myelo	0.5	13.1	88.6	2.9		
	Meta		1.1	9.7	97.1		
	Aty.Lym	1.6				82.4	18.3
	Lym					16.5	79.7
C	Blast	99.4	0.6				
	Pro		91.5	6.6			
	Myelo		7.3	91.0	2.0		
	Meta		0.6	2.4	98.0		
	Aty.Lym	0.6				93.1	5.0
	Lym					6.9	95.0

Second half period

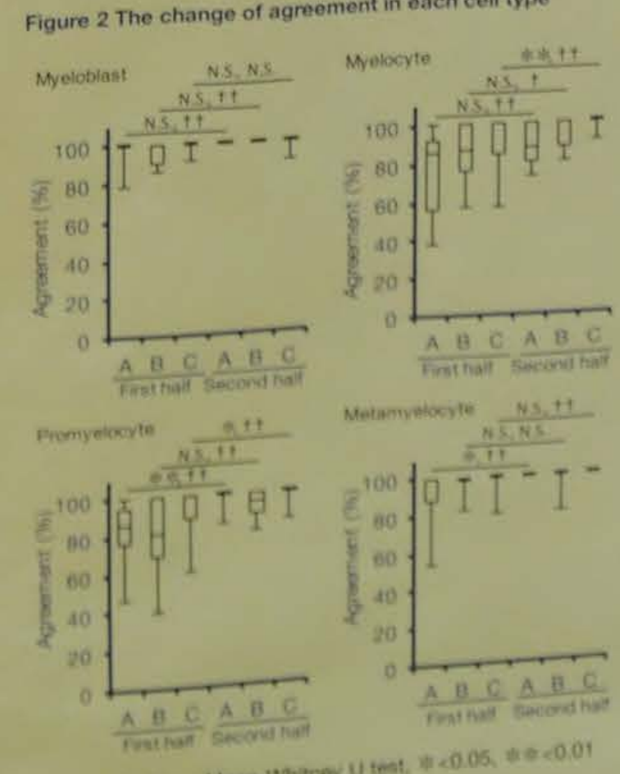
Examiner		JSLH Blast	Pro	Myelo	Meta	Aty.Lym	Lym
A	Blast	100.0					1.0
	Pro		97.2	12.1			
	Myelo		2.8	87.1			
	Meta			0.8	100.0		
	Aty.Lym					95.8	19.4
	Lym					4.2	79.6
B	Blast	100.0	1.2				1.5
	Pro		93.9	1.4			
	Myelo		4.9	94.4	1.5		
	Meta			4.2	98.5		
	Aty.Lym					87.3	17.9
	Lym					12.7	80.6
C	Blast	99.3					
	Pro		98.6	0.8			
	Myelo		1.4	99.2			
	Meta				100.0		
	Aty.Lym	0.7				93.8	3.3
	Lym					6.2	96.7

Blast:Myeloblast, Pro:Promyelocyte, Myelo:Myelocyte, Meta:Metamyelocyte, Aty.Lym:Atypical lymphocyte, Lym: Lymphocyte

Result 3
The change of agreement in each cell type

Agreement in each cell type was compared between first and second half period. In second half period, median agreements of all examiners tended to close to 100%, and its variance tended to shrink, compared with those in first half period.

Figure 2 The change of agreement in each cell type



Median : Mann-Whitney U test, * <0.05, ** <0.01
Variance : F test, † <0.05, †† <0.01
N.S. : Not significance

Conclusion

The IQC calibrates the criteria of evaluation for IG classification, and improved accuracy and repeatability of this test.

(To be continued next presentation)

Hematology

PC-63

Evaluation of an internal QC application for correctly recognizing immature granulocytes on blood smears.

Naoya Ichimura, Ayako Itoi, Yuki Kouda, Yuki Okubo, Michio Hagihara, Syuji Tohda

Clinical Laboratory, Medical Hospital of Tokyo Medical and Dental University

Objectives

We have developed the internal QC (IQC) application for correctly recognizing immature granulocyte (IG) (PC-62 in this conference). This application lets examiners decide the cell type of photo images of IG on computer monitors and quantitatively assesses their performance. It was showed by analysis of IQC results that accuracy and repeatability were improved in IG classification.

The aim of this work is to examine whether IQC using our application improves inter-observer variation of recognition of IG on patients' smears.

Method

The positive samples

Patients' smears including IG were defined as positive samples in each cell type. (e.g. when a test result was myelocyte 1% and metamyelocyte 2% in smear, one as myelocyte-positive and one as metamyelocyte-positive were respectively counted).

In three examiners, patient data of leukocyte differential for one year from June 2014 (period I : n=21,451) , for 10 weeks from 25 Aug 2015 before introducing the application (period II : n=3,125) and after introducing it (period III : n=3,639) were used.

(Refer to a prior presentation regarding work history of examiners)

The evaluation of optically tested samples

Concerning optical tested sample, the proportion of positive samples was calculated every clinical department (e.g. hematology, pediatrics). And, the percentage of clinical department was calculated every examiner.

The change of positives (%)

The percentage of positives were compared between three periods. Multiple comparison was performed using χ^2 test adjusted with Benjamini & Hochberg method .

Inter-examiner variation

Inter-examiner variation using patient data was evaluated by analyzing deviation between expected and observed value. Expected values for the number of positives (E) were defined as following equation.

$$E_{hj} = \sum_{i=1}^{n} (C_{hi} \times P_{ij})$$

h : examiner, i : clinical department, j : cell type,
C : The number of optical tests, P : The proportion of positive samples

The number of positives (O) was counted every examiner and every cell type. The deviation between E and O was statistically analyzed using χ^2 test. Moreover, the change of the deviation from period II to III (D value) was visualized following equation.

$$D = (O - E)^2 / E$$

Result 1
Optically tested sample

The percentage of positives were different every clinical department (Figure 1). That is, prior probabilities of IG positives were different every clinical departments. Moreover, the percentage of clinical departments was different between each examiner (Figure 2). Therefore, we need to consider the difference of clinical department in each examiner so as to obtain a highly accurate expectation. We used upper equation to calculate expect values for positives.

Figure 1 The percentage of positives in each clinical department

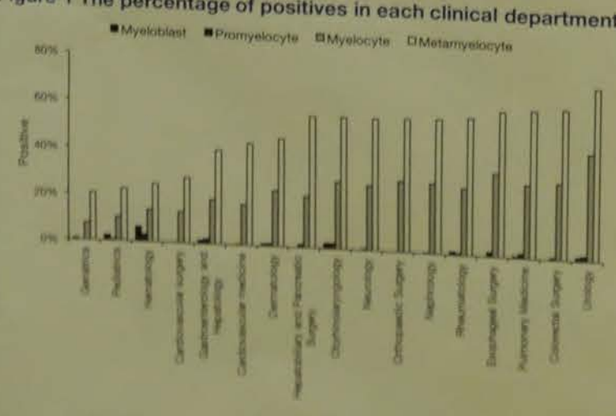
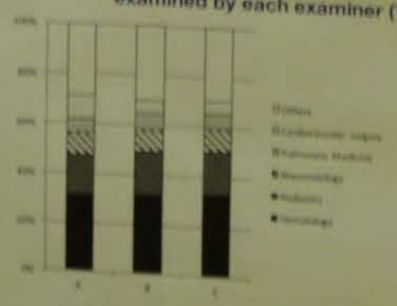


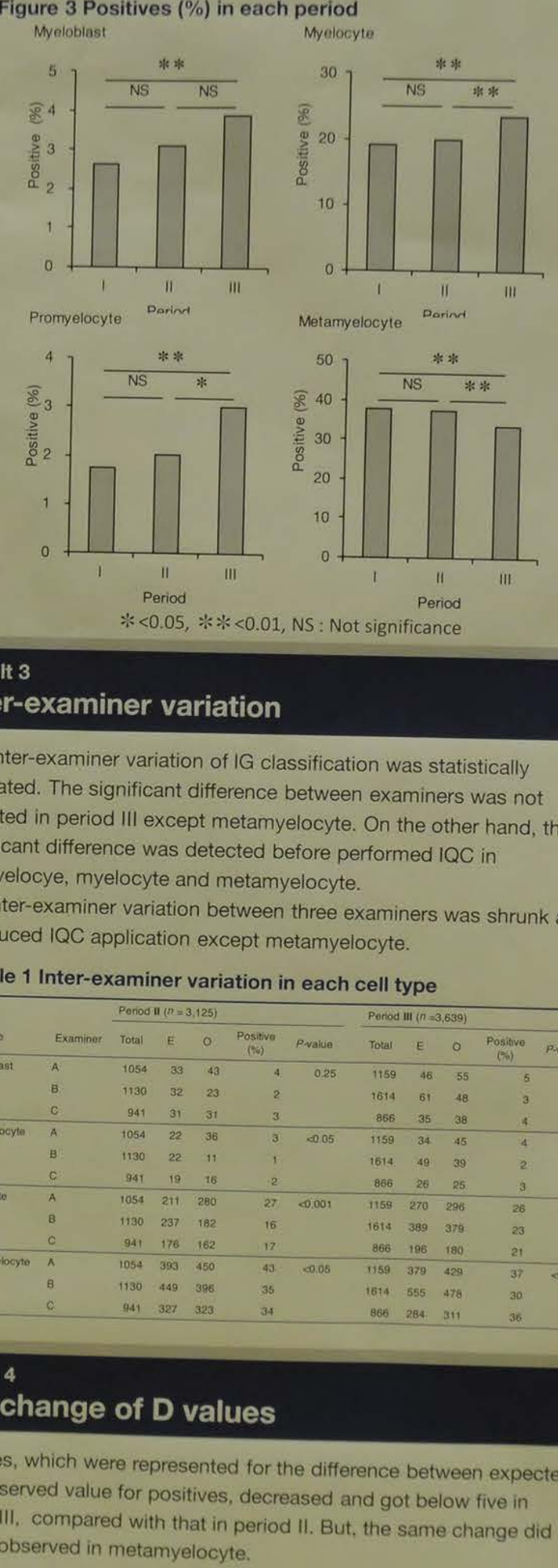
Figure 2 The percentage of clinical departments examined by each examiner (Top 5)



Result 2
The change of positives

Positives were evaluated before and after performed IQC application. The percentage of positives was changed after performed IQC application (period III) in all cell type, but not changed when compared between period I and II. Those values increased except metamyelocyte, but decreased in metamyelocyte.

Figure 3 Positives (%) in each period



*<0.05, **<0.01, NS : Not significance

Result 3
Inter-examiner variation

The inter-examiner variation of IG classification was statistically evaluated. The significant difference between examiners was not detected in period III except metamyelocyte. On the other hand, the significant difference was detected before performed IQC in promyelocye, myelocyte and metamyelocyte.

The inter-examiner variation between three examiners was shrunk after introduced IQC application except metamyelocyte.

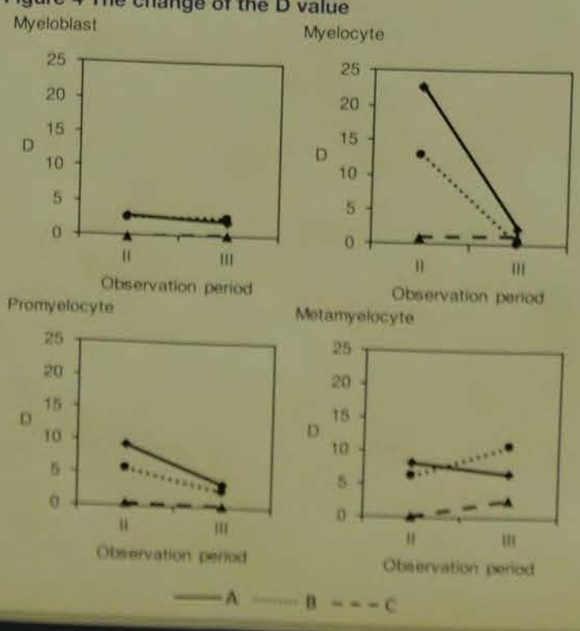
Table 1 Inter-examiner variation in each cell type

Cell type	Examiner	Period II (n = 3,125) Total	E	O	Positive (%)	P-value	Period III (n =3,639) Total	E	O	Positive (%)	P-value
Myeloblast	A	1054	33	43	4	0.25	1159	46	55	5	0.29
	B	1130	32	23	2		1614	61	48	3	
	C	941	31	31	3		866	35	38	4	
Promyelocyte	A	1054	22	36	3	<0.05	1159	34	45	4	0.26
	B	1130	22	11	1		1614	49	39	2	
	C	941	19	16	2		866	26	25	3	
Myelocyte	A	1054	211	280	27	<0.001	1159	270	296	26	0.37
	B	1130	237	182	16		1614	389	379	23	
	C	941	176	162	17		866	196	180	21	
Metamyelocyte	A	1054	393	450	43	<0.05	1159	379	429	37	<0.01
	B	1130	449	396	35		1614	555	478	30	
	C	941	327	323	34		866	284	311	36	

Result 4
The change of D values

D values, which were represented for the difference between expected and observed value for positives, decreased and got below five in period III, compared with that in period II. But, the same change did not be observed in metamyelocyte.

Figure 4 The change of the D value



Discussion

The introduction of the IQC application have led to improve accuracy and repeatability for IG classification on QC data and decrease inter-examiner-variation on patients' data.

The reason why the D value did not decreased in metamyelocyte might be considered that it was not enough to train identification between metamyelocyte and band cell in our IQC application. Therefore, we thought that the result by IQC (refer to a prior presentation) and its by patient data were contradictory to each other in metamyelocyte.

Conclusion

QC implementation improves the inter-examiner variation of IG classification on nts' smears. But, the effect did not be observed in metamyelocyte in this study.

Hematology

PC-64

Validation of inter-laboratory differences and improvements using clinical specimens

Noriko Muraoka [1], Saori Iemata [1], Junko Imai [2], Yuriko Kamimura [1], Hiroshi Kondo [3]

1 Sanki Medical Laboratory , 2 Wakakusa-Daiichi Hospital , 3 Kansai University of Health Sciences

Introduction

The Wakakoukai group has two hospitals and a registered clinical laboratory (S-Lab). They are Wakakusa-Daiichi Hospital (D-Hosp), Wakakusa-Tatsuma Rehabilitation Hospital (T-Hosp), and Sanki Medical Laboratory (Fig. 1). The two hospitals' clinical laboratories usually analyze samples from patients in urgent need of treatment. The S-Lab analyzes other samples. All clinical laboratory results are displayed as time-series data on the network-linked clinical laboratory information system (Fig. 2).

Doctors can check all laboratory results at the three facilities in our group. Therefore, inter-laboratory standardization in common laboratory testing is indispensable. In this study, we validated the inter-laboratory correlation using the laboratory results of the complete blood count (CBC) and leukocyte 5-differential count (5-diff). Furthermore, we attempted to improve the deviation of mean hemoglobin concentration by adjusting automated hematology analyzers based on the laboratory results.

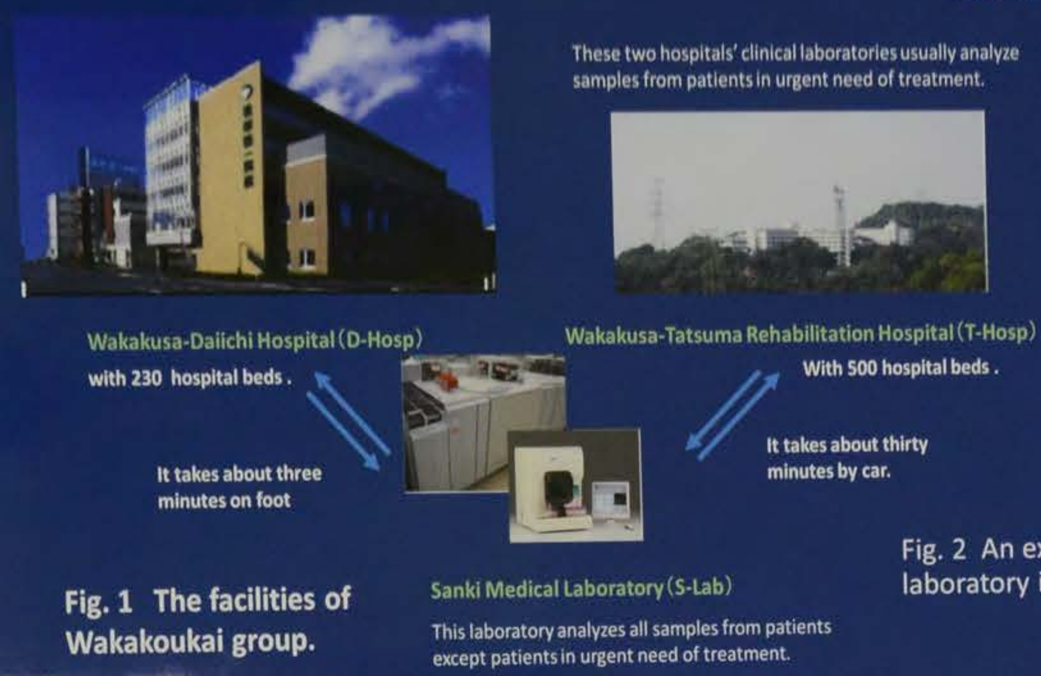


Fig. 1 The facilities of Wakakoukai group.

Fig. 2 An example of the time-series data on the network-linked clinical laboratory information system

Methods

Sysmex XS1000i hematology analyzers were used in the two hospitals, and the Sysmex XT1800i hematology analyzer was used in the S-Lab (Table 1). The e-check type B plus was used as a control sample in the S-Lab, the e-check(XS)type G was used as a control sample in the D-Hosp, and the e-check(XS)type B was used as a control sample in the T-Hosp for internal quality assessment. Peripheral blood samples were drawn from out-patients between 8:30 am and 10:00 am. Five hundred-microliter anti-coagulated blood samples were dispensed into 3 micro-sample tubes (Fig. 3).

The sample tubes were stored in a shipping container with refrigerant and a self-recording thermometer. The shipping containers were sent from the S-Lab to the two hospitals. Sample analysis was duplicated in the manual mode within 4 hours after blood drawing. In the Passing-Bablok regression analysis, it should be confirmed that the 95% confidential intervals (95%CI) of slopes and intercepts include 1.0 and 0.0, respectively; If the range of the population is narrow, Bland-Altman plot analysis should be applied.

Table 1 Automated hematology analyzers and internal quality control samples

	Facilities	Automated hematology analyzers	Internal quality control samples
A	Sanki Medical Laboratory (S-Lab)	XT1800i (Sysmex)	e-CHECK Type B plus
B	Wakakusa-Daiichi Hospital (D-Hosp)	XS1000i (Sysmex)	e-CHECK (XS) Type G
C	Wakakusa-Tatsuma Rehabilitation Hospital(T-Hosp)	XS1000i (Sysmex)	e-CHECK (XS) Type B

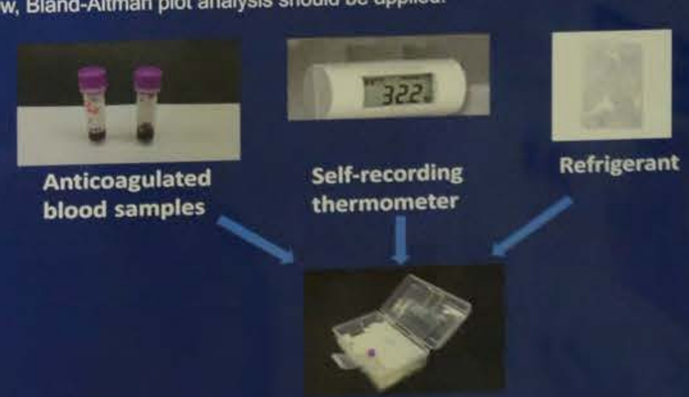


Fig. 3 Shipping condition

Results

The results of internal quality control in S-Lab, D-Hosp and T-Hosp had been stable when this study was conducted (Table 2). Temperature in the shipping container was 14.6 to 26.1 degrees Celsius (Table 3). As one example, The results of the correlation analysis, the Passing-Bablok regression analysis, and Bland-Altman plot analysis of red cell counts between S-Lab and T-Hosp are shown in Figs. 4, 5 and 6, respectively. All results of the correlation analysis, and the Passing-Bablok regression analysis are shown in Tables 4, 5, and 6. Inter-laboratory coefficient of correlation values of CBC were from 0.993 to 0.999 (n=107), and coefficient of correlation values of 5-diff were from 0.671 to 0.998 (n=106). All results of the correlation analysis and the Passing-Bablok regression analysis were excellent. However, the mean hemoglobin concentration of T-Hosp was higher than in the other two facilities. Mean hemoglobin concentrations of S-Lab, D-Hosp, and T-Hosp were 12.4, 12.5, and 12.8 g/dL (n=36), respectively (Table 7). Hemoglobin concentrations of the three facilities converged between 12.9 and 13.0 g/dL (n=45) by adjustment of the automated hematology analyzers.

Table 2 Results of internal quality control samples

		MEAN	SD	CV			MEAN	SD	CV
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

A: S-Lab, B: D-Hosp, C: T-Hosp

Table 3 Temperature in the shipping container

Days	S-Lab	D-Hosp	T-Hosp
1	21.8	21.9	22.2
2	22.2	23.9	22.9
3	25.0	24.2	23.5
4	25.3	21.7	22.1
5	23.8	18.7	19.4
6	18.0	26.1	25.4
7	17.8	17.7	18.6
8	22.2	19.6	15.8
9	22.2	19.6	16.4
10	20.6	19.4	17.8
11	20.3	19.6	18.9
12	17.1	18.0	18.2
13	16.8	18.1	16.7
14	16.9	18.5	17.9
15	17.9	15.0	14.6
16	15.4	18.9	18.6
17	18.4	21.2	18.2

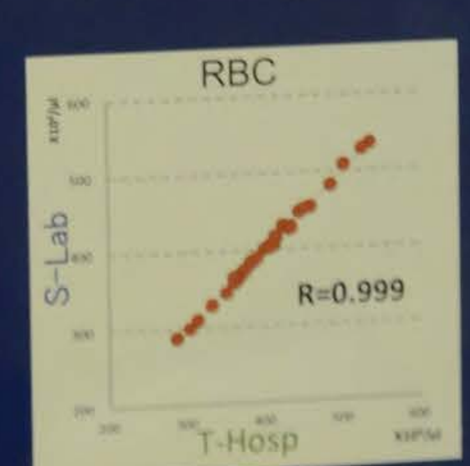


Fig. 4 Correlation

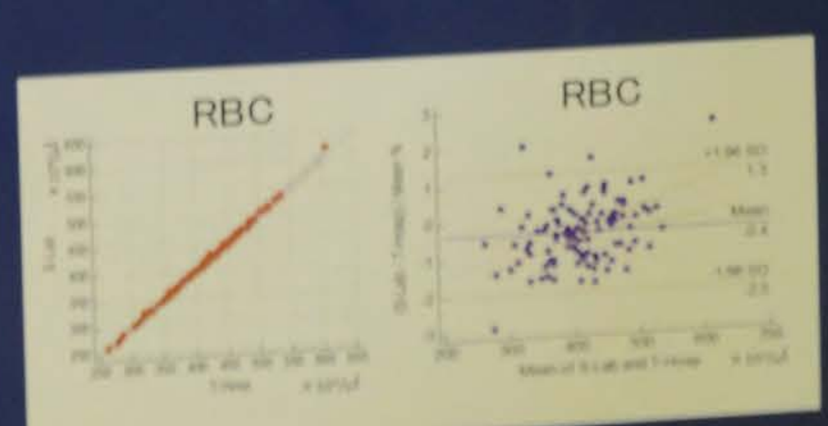


Fig. 5 Passing and Bablok regression

Fig. 6 Bland-Altman plot

Table 4 Results of correlation analysis and Passing Bablok regression analysis of blood cell counts between S-Lab and D-Hosp

	Correlation		Passing and Bablok regression				
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Table 5 Results of correlation analysis and Passing Bablok regression analysis of blood cell counts between S-Lab and T-Hosp

	Correlation		Passing and Bablok regression				
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Table 6 Results of correlation analysis and Passing Bablok regression analysis of blood cell counts between D-Hosp and T-Hosp

	Correlation		Passing and Bablok regression				
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Table 7 Improvement of the deviation of mean hemoglobin concentrations by adjustment of automated hematology analyzers

	N	S-Lab	D-Hosp	T-Hosp
Pre-adjustment	36	12.40±1.95	12.52±1.97	12.83±1.99
Post-adjustment	45	12.94±1.86	12.95±1.84	12.99±1.84

Conclusion

Validation of the inter-laboratory correlation could be carried out smoothly using clinical specimens. The results of correlation analysis proved useful to improve the deviation of hemoglobin concentrations.

Hematology

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The effect of adiponectin on human eosinophil functions: a possible mechanism for relationship between obesity and asthma

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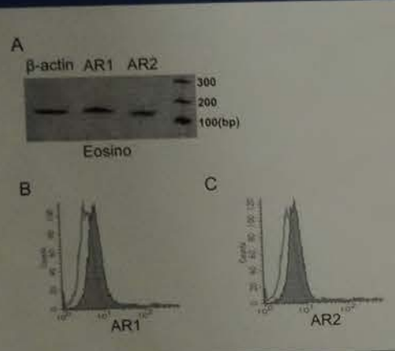
Objective

The aim of our study was to examine whether adiponectin might affect functions and intracellular signaling of eosinophils, which play an important role in the pathogenesis of asthma.

Background

Obesity is associated with asthma, in terms of increased prevalence, reduction in lung functions, and reduced response to medication. Adiponectin, an adipocyte-derived cytokine, is known to have anti-inflammatory effects with reduced concentrations in obese subjects. Recent findings raised the intriguing possibility that adiponectin might play a role in allergic inflammation, although the mechanistic basis for their relationship remains unclear.

Results I: Expression of adiponectin receptors on human eosinophils

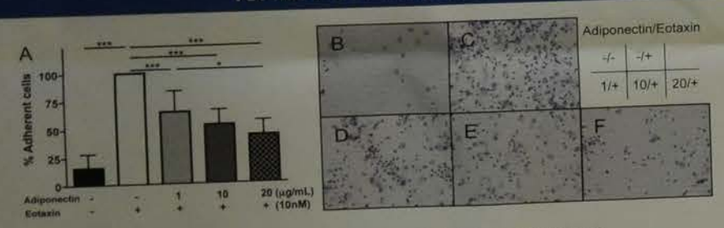


(A) The mRNA expression profile in freshly isolated human eosinophils was studied using RT-PCR. The amplified products were separated on a 12.5% polyacrylamide gel, and the gel was subjected to silver staining. AdipoR1 (AR1, 170 bp) and AdipoR2 (AR2, 156 bp) mRNA is expressed in purified eosinophils (A). β-actin (189 bp) was used for loading control.

(B, C) Purified peripheral blood eosinophils were stained with anti-AdipoR1 Ab or anti-AdipoR2 Ab, and then subjected to flow cytometric analysis.

Both AdipoR1 and AdipoR2 were expressed in human eosinophils.

Results II: Effect of adiponectin on eosinophil adhesion to ICAM-1-coated plates

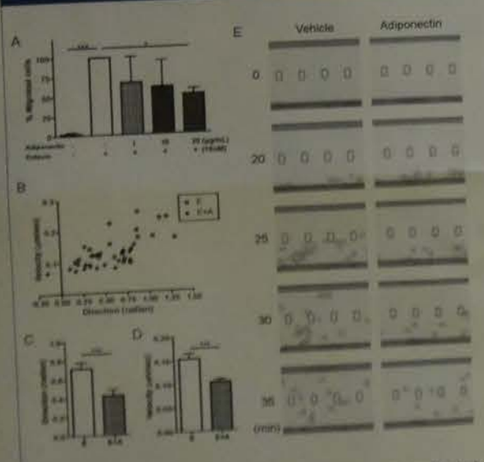


(A) Purified eosinophils were pretreated with or without indicated concentrations of adiponectin for 60 min and then transferred to ICAM-1-coated plates in the presence or absence of eotaxin and incubated for 60 min for eosinophil adherence. The numbers of adherent eosinophils were counted by light microscopy. The results are expressed as the relative number of eotaxin-stimulated adherent cells with a value of 100%. Data are expressed as the mean ± SD from five different donors. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, repeated measures ANOVA with Bonferroni correction.

(B-F) Representative images of eosinophil adhesion to ICAM-1-coated plates.

Eotaxin-enhanced adhesion was inhibited by pretreatment with adiponectin.

Results III: Effect of adiponectin on eotaxin-directed eosinophil chemotaxis

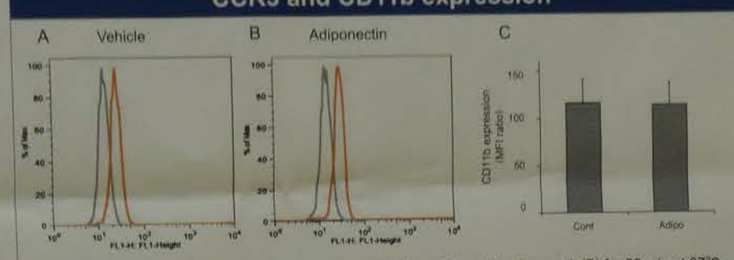


(A) Purified eosinophils were pretreated with or without indicated concentrations of adiponectin for 60 min, and eotaxin-induced migration assays were performed using Boyden chambers. Eosinophils were placed in the upper wells, and chemotactic responses to eotaxin in lower wells were studied. The loaded chambers were incubated for 60 min. Data are expressed as the mean of five experiments ± SD from different donors. $^{*}p < 0.05$, $^{***}p < 0.001$, repeated measures ANOVA with Bonferroni correction.

(B) Velocity-Directionality plot (VD plot) of adiponectin-pretreated cells. Chemotaxis toward the concentration gradient of eotaxin was assessed using a real-time horizontal microchannel device (TAXIScan). Twenty-one cells were tracked, and mean velocity and directionality of each cell were calculated using TAXIScan Analyzer 2 software. Vehicle-treated cells migrating toward eotaxin (E) are expressed as open circles, and adiponectin-pretreated cells (E+A) are expressed as filled diamond shapes. (C) Comparison of cell directionality between vehicle and adiponectin. Data are expressed as the mean of 21 cells ± SD. $^{***}p < 0.001$, Wilcoxon matched-pairs signed-ranks test. (D) Comparison of cell velocity between vehicle and adiponectin. Data are expressed as the mean of 21 cells ± SD. $^{***}p < 0.001$, Wilcoxon matched-pairs signed-ranks test. (E) Representative images of eotaxin-directed migration using TAXIScan system. Cells were applied to one compartment of the holder and eotaxin was applied to the opposite. Chemotaxis toward the concentration gradient of eotaxin (from top to bottom in the figure) was inhibited by adiponectin pretreatment.

Adiponectin diminished eotaxin-directed chemotactic responses by deluting velocity and directionality.

Results IV: The effect of adiponectin on CCR3 and CD11b expression

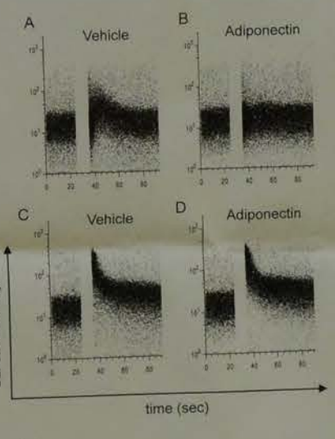


(A, B) Purified eosinophils were incubated with vehicle(A) or 20 μg/mL adiponectin(B) for 60 min at 37°C, after which cells were stained with FITC-conjugated anti-human CCR3 mAb or isotype-matched control mAb. The stained cells were analyzed using a flow cytometer. The expression of CCR3 was assessed as the ratio of the MFI of the sample and the isotype-matched control (n=3).

(C) Purified eosinophils were incubated with vehicle or 20 μg/mL adiponectin for 60 min at 37°C, after which cells were stained with FITC-conjugated anti-human CD11b mAb or isotype-matched control mAb. The stained cells were analyzed using a flow cytometer. The expression of CD11b was assessed as the ratio of the MFI of the sample and the isotype-matched control (n=3).

Adiponectin did not affect eosinophil CCR3 and CD11b expression.

Results V: The effect of adiponectin on eotaxin-elicited intracellular calcium influx



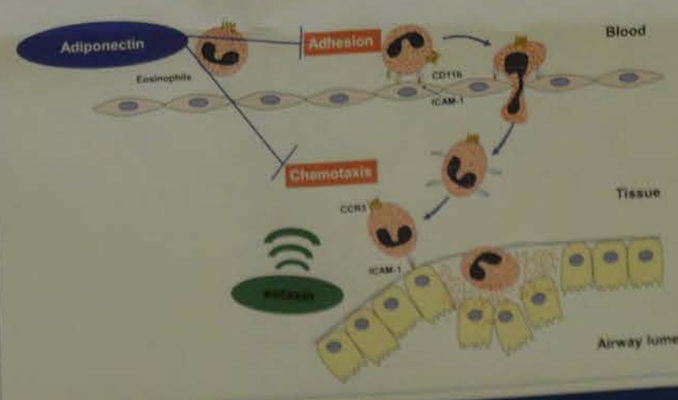
Eosinophils were incubated with vehicle (A, C) or 20 μg/mL adiponectin (B, D) for 60 min and then loaded with calcium-sensitive dye Cal-520. The changes in intracellular free calcium levels induced by different concentrations of eotaxin were detected as the increase in the fluorescence intensity of Cal-520. Response to 0.1 nM eotaxin (A, B) and 50 nM eotaxin (C, D) was studied by flow cytometry. The results are representative of three independent donors with similar results.

(E) Purified eosinophils were incubated with vehicle or 20 μg/mL adiponectin for 60 min at 37°C, after which cells were stained with FITC-conjugated anti-human p-ERK mAb or isotype-matched control mAb. The stained cells were analyzed using a flow cytometer. The expression of p-ERK was assessed as the MFI of the sample (n=3).

Calcium influx in response to eotaxin was attenuated by adiponectin, although p-ERK expression was not.

Conclusions

The series of our study indicated that adiponectin attenuated the eosinophil chemotaxis and adhesion induced by eotaxin through modification of calcium signaling. These findings provide the evidence for the previously unrecognized mechanisms of direct interaction between adipocytokine and eosinophil functions.



References

Hematology

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基隆醫院

Improving Laboratory Quality for Single Hematocrit Order on Current Dialysis Anemia Monitoring in Taiwan

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Purpose

In Taiwan dialysis treatment followed DRG system. Clinician followed the practices from Ministry of Health and Welfare, Taiwan Society of Nephrology, they ordered single HCT (Hematocrit) out of full CBC for anemia monitoring. Single HCT could lead misjudgments of anemia on a case. We made corrective actions.

Materials and Methods

A 59 y/o woman underwent dialysis treatment. On 2015-Sep-9, HCT32.7% . Sep-22, HCT28.7% . On Oct-8, her HCT dropped to 23% . We doubted and rechecked, and found MCHC53.9g/dL. With unreasonable MCHC, blood appearance looked like sands, these tallied with cold agglutination and falsely low HCT. Under microscope RBCs agglutinated, cold agglutination test 1:128 (1+). By using plasma replacement and water bath (*37°C/30 min*), *no more agglutination* under microscope, then examined CBC immediately on the sample from bath.

Results

On Oct-8 before pre-treatment Hb12.4g/dL, HCT23% and MCHC53.9g/dL. After pretreatment Hb12.8g/dL, HCT37.6% and MCHC34g/dL. Cold agglutination was confirmed.

Conclusion

MCHC (Hb/HCT ratio) is commonly used to judge erroneous results. But if the order is only HCT, LIS only collects HCT. With insufficient information, MTs could report HCT wrongly. After discussion we made 2 actions. The first, combine Hb and HCT for the order in our hospital. This will help clinician to evaluate the anemia condition. There's no great repulsion for the other examinations under the DRG system. The second, LIS transforms the one-item to full CBC order for analyzer. This will help MTs to judge the results. There's no difference between single item and full CBC on our analyzer.

Figure 1、Before Plasma Replacement

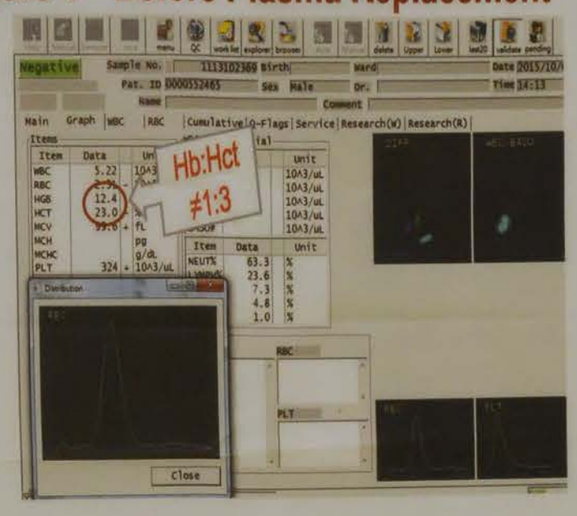

Figure 2、Before Plasma Replacement

Figure 3、After Plasma Replacement

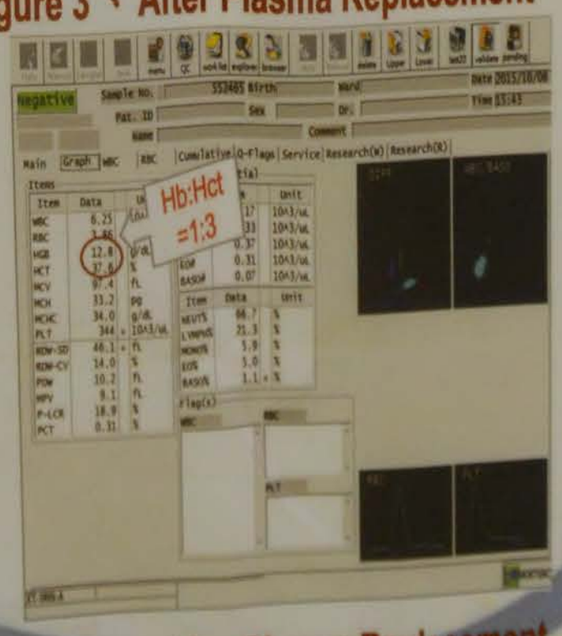

Figure 4、After Plasma Replacement

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Kobe, Japan, Aug 31-Sept 04, 2016

PENTADECAPEPTIDE BPC 157 AND ITS EFFECTS ON HEMOSTATIC PARAMETERS IN RATS TREATED WITH WARFARIN, L-NAME AND L-ARGININE

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INTRODUCTION

BPC 157 is a stable gastric pentadecapeptide recently implicated with a role in hemostasis. As a particular anti-ulcer peptide (GEPPPGKPADDAGLV, M.W. 1419, PL-10, PLD-116, PL 14736) **Fig.1**, in trials for inflammatory bowel disease, wound treatment, producing no reported toxicity (LD1 could be not achieved), effective alone without carrier), it has a particular effect on endothelium protection along with prominent angiogenesis and an effect of NO-system and endothelin serum level (that may have a particular role in bleeding disturbances)[1].

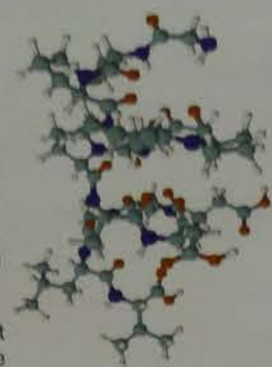

Fig.1. BPC 157 molecular structure 3D.

We focused on the counteraction of the effect on different anticoagulants in treated rats undergoing amputation, particularly the reduction of bleeding time and the lower incidence of thrombocytopenia after amputation in animals receiving the BPC 157 [2].

Furthermore, nitric oxide (NO) is an endogenously produced vasodilator which is a potent regulator of vascular homeostasis. While NO is largely implicated in hemostatic mechanisms, in tail-amputation-models under anticoagulants administration, both the NO-synthase (NOS)-blocker, L-NAME (prothrombotic) and the NOS-substrate L-arginine (antithrombotic), were little investigated. Thus, we investigate the effect of BPC 157, L-NAME and L-arginine on hemostatic parameters under different pathological condition (tail amputation without or with anticoagulants)[3].

OBJECTIVE

This study determines the effect of the pentadecapeptide BPC157 on the values of hemostatic parameters in rats after administration of anticoagulant warfarin. Also, this study extends and clarifies the effect of BPC 157 on hemostasis with addition of N(G)-nitro-L-arginine methylester (L-NAME) and L-arginine in warfarin treated rats.

MATERIALS AND METHODS

Animals

Male Albino Wistar rats were used in all of the experiments (10 rats per experimental group and interval). The study was approved by the Local Ethics Committee and experiments were assessed by observers unaware of the given treatment.

Drugs

Medication, without carrier or peptidase inhibitor, included stable gastric pentadecapeptide BPC 157 (a partial sequence of the human gastric juice protein BPC, freely soluble in water at pH 7.0 and in saline). It was prepared as a peptide with 99% (HPLC) purity (1-des-Gly peptide was the main impurity; manufactured by Diagen, Ljubljana, Slovenia) in dose and application regimens as described before [2,4,5]. Accordingly, warfarin (Martefarin (Orion Pharma, Finland)) dissolved in saline, L-NAME (Sigma, USA) and L-arginine (Sigma, USA) dissolved in saline were used as well [2,3,4,5].

Bleeding procedures, diagnostic tests

Platelet count, hematocrit, prothrombin time, active partial thromboplastin time, thrombin time, fibrinogen and bleeding, were measured to evaluate the hemostatic effect of the agents. The blood samples assessment was carried precisely as described before [2,3] (**Table 1**).

Bleeding was monitored as described before [2,3] until blood flow stopped for a complete 30-s interval or till the end of 60 min period. In deeply anaesthetised rats (placed in ventral position) the tail was transected with a surgical scalpel 3 cm from the tip (and submerged into a tube with 15 ml of saline at room temperature in vertical position) and the duration and amount of the bleeding were measured to evaluate the hemostatic effect of the agents or saline administration (**Fig.2**).

Fig.2. Measurement of bleeding time and amount of bleeding (tail amputation in rats)

Statistical analyses

Statistical analyses of the quantified data were performed by analysis of variance (ANOVA). Post-hoc comparisons were appraised using the conservative Bonferroni/Dunn test. Data are presented as the mean ± standard deviation (SD). Values of P<0.05 were considered statistically significant.

Table 1. The blood samples assessment, laboratory methods used in the study.

Parameters	Methods/Reagents	Optimum specimens/ Tested within	Analyzers	Units	Normal range*
Prothrombin time (PT)	Performed according to the technique by Quick using human placental thromboplastin (Thromborel® S, Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany)	Plasma/ 2 hours	Automated coagulation analyzer BCS® XP System (Siemens, Marburg, Germany)	ratio	1.25-1.45
Activated partial thromboplastin time (APTT)	Performed by using Actin FS (Dade® Actin® FS APTT Reagent, Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany) containing the purified soy phosphatides	Plasma/ 2 hours	Automated coagulation analyzer BCS® XP System (Siemens, Marburg, Germany)	ratio	0.38-0.51
Thrombin time (TT)	Performed according to the instructions from manufacturer using bovine thrombin (BC Thrombin Reagent, Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany)	Plasma/ 2 hours	Automated coagulation analyzer BCS® XP System (Siemens, Marburg, Germany)	sec	30-50
Fibrinogen concentration (FIB)	Determined by a modification of Clauss' coagulometric method using bovine thrombin (Multifibren® U, Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany)	Plasma/ 2 hours	Automated coagulation analyzer BCS® XP System (Siemens, Marburg, Germany)	g/L	1.5-2.0
Platelet count (PLT)	Performed by using Coulter principle (impedance) (Beckman Coulter Reagents)	Whole blood/ 2 hours	Automated Coulter® HmX Hematology Analyzer (Beckman Coulter Inc., Florida, USA)	10^9/L	650-1100
Hematocrit (HTC)	Performed by directly measuring from RBC histogram	Whole blood/ 2 hours	Automated Coulter® HmX Hematology Analyzer (Beckman Coulter Inc., Florida, USA)	L/L	0.38-0.49

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RESULTS

In rats, after (tail) amputation, with or without warfarin (1.5 mg/kg i.g. once daily for 3 consecutive days), BPC 157 (10 µg/kg, 10 ng/kg, i.p./i.g.) reduced bleeding time and/or haemorrhage and counteracted thrombocytopenia.

Furthermore, the effect of L-NAME and L-arginine on hemostatic parameters, and the effects of BPC 157 on the L-NAME- and L-arginine-induced hemostatic actions under different pathological condition: tail amputation without or with anticoagulant warfarin are shown in **Table 2** and **Table 3**. As for L-NAME and/or L-arginine, we noted: L-arginine (100 mg/kg i.p.)-rats: more bleeding, less/no thrombocytopenia; L-NAME (5 mg/kg i.p.)-rats: less bleeding (amputation only), but present thrombocytopenia; L-NAME+L-arginine-rats also exhibited thrombocytopenia: L-NAME counteracted L-arginine-increased bleeding, L-arginine did not counteract L-NAME-counteracted thrombocytopenia. All of the animals receiving BPC 157 in addition (BPC 157µg+L-NAME; BPC 157µg+L-arginine, BPC 157µg+L-NAME+L-arginine), exhibited decreased haemorrhage and markedly counteracted thrombocytopenia.

In addition, platelet counts were corrected with hematocrit to avoid errors that may be caused by animals bleeding, and were expressed as a percentage of baseline for each time point of the tested groups. The hematocrit-corrected platelet counts are shown in **Fig.3**.

Table 2. Normal (amputation only) rats. Tail amputation, bleeding time, amount of bleeding, PLT, HCT, PT, APTT, TT, FIB, values in normal rats with amputation challenged with BPC 157 (10 µg/kg, 10 ng/kg), L-NAME (5 mg/kg), L-arginine (100 mg/kg) given alone and/or together, applied intraperitoneally, at 30 minutes before amputation while controls received simultaneously an equivolume of saline (5ml/kg intraperitoneally). Mean ± SD, *P<0.05 at least vs. control.

NORMAL	Bleeding Time	Bleeding Amount	PLT before	PLT after	HCT before	HCT after	PT	APTT	TT	FIB
(Tail amputation)	min	ml	x10⁹/L	x10⁹/L	L/L	L/L	ratio (PR)	ratio	sec	g/L
Control	18± 3	1.92± 1.1	853± 173	605± 35	0.446± 0.03	0.430± 0.04	1.30± 0.22	0.45± 0.16	34± 12	1.6± 0.22
BPC µg	7± 2*	0.21± 0.06*	820± 167	685± 22*	0.461± 0.03	0.453± 0.03	1.32± 0.25	0.41± 0.17	40± 11	1.8± 0.17
BPC ng	8± 2*	0.24± 0.04*	831± 178	675± 17*	0.454± 0.04	0.446± 0.03	1.35± 0.21	0.42± 0.11	41± 13	1.7± 0.19
L-arginine	29± 4*	1.63± 1.2	781± 123	702± 30*	0.497± 0.03	0.476± 0.03	1.29± 0.21	0.64± 0.18	35± 9	1.8± 0.23
L-NAME	7± 2*	0.75± 0.15*	677± 105*	601± 40*	0.481± 0.04	0.454± 0.03	1.28± 0.19	0.66± 0.21	30± 8	1.8± 0.18
L-NAME+ L-arginine	21± 3*	0.8± 0.24*	695± 94*	442± 15*	0.482± 0.05	0.468± 0.03	1.24± 0.22	0.51± 0.13	46± 14	1.8± 0.21
L-arginine+ BPC 157 µg	9± 2*	0.57± 0.11*	801± 189	694± 39*	0.506± 0.04	0.490± 0.04	1.28± 0.21	0.49± 0.17	39± 11	1.8± 0.27
L-NAME+ BPC 157 µg	8± 2*	0.40± 0.12*	758± 122	654± 25*	0.472± 0.03	0.465± 0.04	1.30± 0.22	0.49± 0.18	40± 10	1.8± 0.19
L-NAME+L-arginine +BPC 157 µg	10± 3*	0.30± ±0.09*	788± 133	655± 31*	0.479± 0.03	0.466± 0.03	1.34± 0.23	0.5± 0.13	48± 13	1.8± 0.18

Table 3. Warfarin rats. Tail amputation, bleeding time, amount of bleeding, PLT, HCT, PT, APTT, TT, FIB values in warfarin rats. Warfarin was given intragastrically (1.5 mg/kg) once daily for 3 consecutive days, with the last challenge was at 3 hours before the bleeding procedure. BPC 157 (10 µg/kg, 10 ng/kg) was given immediately after any warfarin challenge, intragastrically while controls received simultaneously an equivolume of saline (5.0 ml/kg intragastrically). We applied L-NAME (5 mg/kg), L-arginine (100 mg/kg) alone or combined, intraperitoneally, at 30 minutes before amputation. Mean ± SD, *P<0.05 at least vs. control.

WARFARIN	Bleeding Time	Bleeding Amount	PLT before	PLT after	HCT before	HCT after	PT	APTT	TT	FIB
(Tail amputation)	min	ml	x10⁹/L	x10⁹/L	L/L	L/L	ratio (PR)	ratio	sec	g/L
Control	> 60	6.58± 1.2	612± 72	302± 79	0.441± 0.04	0.364± 0.02	<0.05	2.86± 0.42	62± 6	1.5± 0.15
BPC µg	48.6± 15*	5.52± 1.4*	693± 68*	456± 116*	0.461± 0.03	0.417± 0.02*	<0.05	2.81± 0.38	60± 7	1.7± 0.25
BPC ng	> 60	5.65± 1.1*	706± 88*	418± 105*	0.465± 0.02	0.420± 0.03*	<0.05	2.83± 0.47	58± 8	1.5± 0.21
L-arginine	> 60	7.8± 1.4*	638± 89	525± 98*	0.521± 0.06	0.455± 0.04	<0.05	2.03± 0.36	59.9± 8	1.9± 0.19
L-NAME	> 60	8.0± 1.8	654± 49*	346± 59	0.454± 0.03	0.413± 0.03	<0.05	2.60± 0.39	62.6± 7	1.8± 0.17
L-NAME+ L-arginine	> 60	7.0± 1.7	483± 87*	283± 35	0.467± 0.04	0.415± 0.03	<0.05	2.68± 0.34	60.6± 8	1.8± 0.21
L-arginine+ BPC 157 µg	> 60	4.9± 0.6*	650± 105	514± 119*	0.476± 0.04	0.472± 0.04	<0.05	2.03± 0.38	59.8± 8	1.6± 0.17
L-NAME+ BPC 157 µg	> 60	5.0± 0.9*	637± 88	461± 105*	0.475± 0.04	0.415± 0.02	<0.05	1.74± 0.29	60.1± 7	1.6± 0.15
L-NAME+L-arginine +BPC 157 µg	> 60	4.5± 0.7*	612± 79	493± 113*	0.501± 0.05	0.481± 0.03	<0.05	1.92± 0.31	78.3± 12	1.8± 0.18

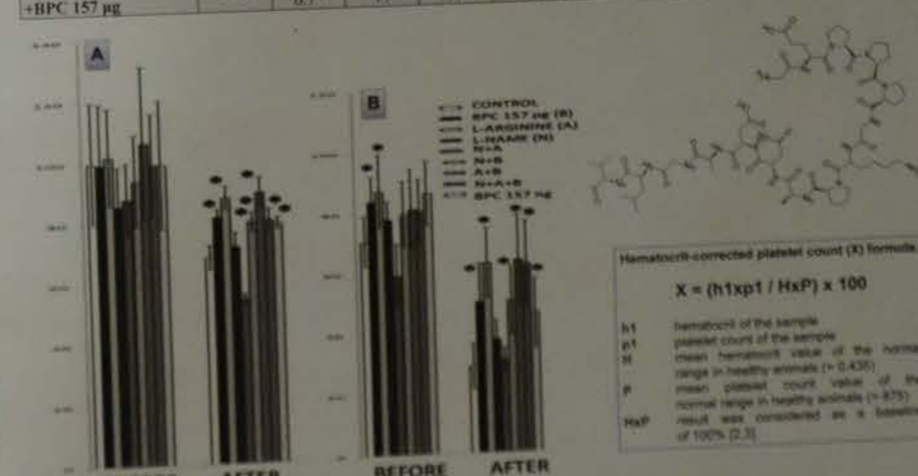

Fig. 3. Hematocrit-corrected platelet counts (X) before, and after bleeding period in: (A) normal, (B) warfarin challenged rats when treated with BPC 157 (10 µg/kg, 10 ng/kg), L-NAME (5 mg/kg), L-arginine (100 mg/kg) alone or combined. Mean ± SD, *P<0.05 at least vs. control.

Acknowledgments

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References

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CONCLUSIONS

BPC 157 has an effect on hemostatic parameters, especially on bleeding and platelet count, in treated rats. L-NAME (thrombocytopenia), L-arginine (increased haemorrhage) counteraction and BPC 157 (decreased haemorrhage, counteracted thrombocytopenia) with rescue against anticoagulant (warfarin), implicate a BPC 157 …dulatory and balancing role with rescued NO-hemostatic mechanisms.