Urinalysis

PH-01

Phagocytosis Phenomenon in Urinary Tract of a Poor Glycemic Control Patient A Case Report and Survey

Hui-Szu Tsai, Chuan-Po Lee, Ya-Chin Li, Hsiu-Chin Fan*

Division of Quality Management, Department of Pathology & Laboratory Medicine, Taipei Veterans General Hospital, Taiwan, R.O.C.

Introduction

Cytoplasmic inclusions are sometimes seen in cells in urinary sediment. The size and shape of the inclusions vary. They could be smaller than an RBC or larger than a WBC. They could be round, oval, ring form or irregular in shape. They are dark purple in Sternheimer stain. In this study, we intended to find out the occurrence rate of inclusion in hospital-based urine specimens as well as its relationship with the parameters of urine routine examination.

Case Profile

A 73-year-old man was sent to our emergency department due to general muscle weakness and drowsiness for one day after head injury in a bicycle accident. After examinations, he was found to have an acute right centrum semiovale infarction, urinary tract infection (UTI) and chronic kidney disease (CKD). He also had hypertension, hyperlipidemia and type II DM. His serum glucose level was 434 mg/dl and the urine glucose was 1.0 mg/dl, indicating that his DM condition was poorly controlled. BUN 19 mg/dl, creatinine 1.53 mg/dl, eGFR 45 ml/min/1.73 m^2, urine protein (PRO) 30 mg/dl supported the diagnosis of CKD. Urine occult blood (OB) 3+, nitrite (NIT) +, leukocyte esterase (LEU) 2+, RBC 11~20/HPF, WBC 1+/HPF, and many bacteria (cultured as normal flora) showed this patient also had an active urinary tract infection (Table 1). High CRP with normal lymphocyte, monocyte in DC might rule out the possibility of viral infection. In the urine sediment, we also observed many cytoplasmic inclusion bodies (yellow arrow) and vacuoles of phagocytosis reaction (green arrow) (Figure 1).

Table 1. Laboratory Data of Subject.

Chemistry Strip						Sediment
Color	Clarity	GLU	BIL	KET	SG	RBC: 11~20 / HPF WBC: 1+ / HPF Renal tubular epithelial cell (RTE): 0~2 / HPF Atypical cell: 0~2 / HPF Bacteria many (cultured as normal flora) Intracellular inclusion body present
Yellow	1+	1.0 g/dl	-	-	1.030	
OB	pH	PRO	URO	NIT	LEU	
3+	5.5	30 mg/dl	1.0 E.U./dl	+	2+	

CBC, DC	WBC ($\times10^3/\mu L$)	RBC ($\times10^6/\mu L$)	Hb (g/dL)	PLT ($\times10^3/\mu L$)	Neu. (%)	Lym. (%)	Mo. (%)	Eo. (%)	Baso. (%)
Result	7.8	4.5	12.4	278	70.1	19.2	8.6	1.7	0.4
Reference	4.5~11	4.2~5.4	14~18	150~450	47~75	20~45	0~9	0~8	0~1

Chemistry	BUN (mg/dL)	Crea (mg/dL)	GLU (mg/dL)	24hr Urine Crea (mg/day)	T. Bil (mg/dL)	TG (mg/dL)	CRP (mg/dL)
Result	19	1.53	434	45.70 (2,200 ml)	0.76	107	3.01
Reference	7~20	0.5~1.2	61~115	1.0~2.0 g/day	0.2~1.6	20~200	0~0.5

rule out the viral infection, while urine culture resulted in normal flora positive. Suboptimal urine specimen like delay delivery may have bacteria contamination, cytoplasmic inclusion body is worthy in such situation to differentiate from true infection. For RBC present cases, isomorphic RBC is relative to macrophage while dysmorphic RBC is relative to renal tubular cell.

Table 2. Discovery Rate in Different Parts of Specimens.

Parts	OPD	IPD	ER	Total
Specimen volume	6,741	3,454	1,763	11,958
Inclusion body Present	106	64	49	219
Discovery rate (%)	1.57%	1.85%	2.78%	1.83%

Table 3. Relations of Inclusion Body with Urinalysis Results.

Parameter (11,958)	GLU	OB	PRO	NIT	LEU	RBC*	WBC**
Specimen	1,411	5,212	4,201	896	3,823	3,887	2,842
Inclusion body present	28	174	142	79	177	160	166
P value***	0.6481	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Discovery rate	1.98%	3.34%	3.38%	8.82%	4.63%	4.12%	5.84%

Parameter (11,958)	RBC* & WBC** Co-exist	Bacteria	Yeast	Bacteria & Yeast Co-exist
Specimen	1,772	2,487	257	147
Inclusion body present	132	139	13	11
P value***	<0.001	<0.001	<0.001	<0.001
Discovery rate	7.45%	5.59%	5.06%	7.48%

*RBC > 2/HPF, **WBC > 5/HPF, ***Spearman rank correlation coefficient.

Table 4. Analysis of 219 Inclusion Body Present Cases

Strip (219)	GLU	BIL	KET	OB	PRO	URO	NIT	LEU
Inclusion body present	28	36	48	174	142	2	79	177
Discovery rate	12.8%	16.4%	21.9%	79.5%	64.8%	0.9%	36.1%	80.8%

Sediment (219)	RBC*	WBC**	Renal tubular cell	Urothelial cell	Bacteria	Yeast
Inclusion body present	160	166	101	54	139	13
Discovery rate	73.1%	75.8%	46.1%	24.7%	63.5%	5.9%

*RBC > 2/HPF, **WBC > 5/HPF.

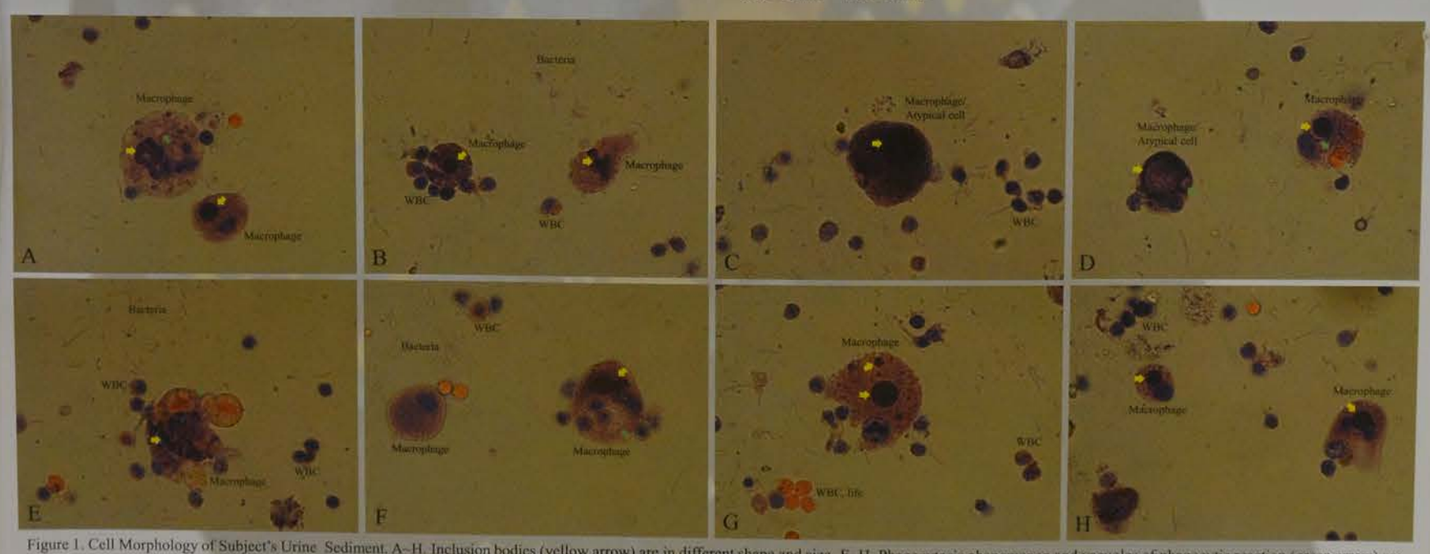


Figure 1. Cell Morphology of Subject's Urine Sediment. A~H. Inclusion bodies (yellow arrow) are in different shape and size. E~H. Phagocytosis phenomenon and vacuoles of phagocytic reaction (green arrow) are showed. (400X, Modified Sternheimer Stain,TOYOBO)

Survey

From 11,958 urinalysis specimens (OPD 6,741, IPD 3,454, ER 1,763) that we collected during a 30-day period (2016.02.22~2016.03.22), 219 (1.83%) specimens had cytoplasmic inclusions. It was more commonly seen in the ER specimens (2.78%; Table 2). The correlation of cytoplasmic inclusion and the other urinalysis results are listed in Table 3. Inclusions were more commonly associated with NIT positive, RBC and WBC coexist, as well as bacteria and yeast coexist, and the discovery rates of inclusions among these parameters were 8.82%, 7.45% and 7.48%, respectively (P<0.001). However, nitrite positive is known as the character only for Gram (-) bacilli. Of the 219 cases that had cytoplasmic inclusions, the relationship of inclusions with the other parameters are listed in Table 4. Significant correlations were noted in for clinical care. LEU, OB, and bacteria present, with the association rates were 80.8%, 79.5% and 63.5%, respectively.

Discussion

Cytoplasmic inclusions could be the results of viral infection or cellular endocytosis (Figure 1 & 2). They could be seen in macrophage and urinary tract epithelium, e.g., renal tubular cells and urothelial cells. However, in many cases, the nature of the inclusion-harbored cells could not be distinguished as the original cellular structures could be transformed. As LEU, OB, and bacteria present are parameters for urinary tract infection (UTI), significant correlation between these parameters and cytoplasmic inclusions indicated that the latter could also be used as an indicator for UTI. It is known patients of DM or CKD might be immunocompromised and have higher risk for infections. The lab data of present subject showed high CRP with normal DC that might

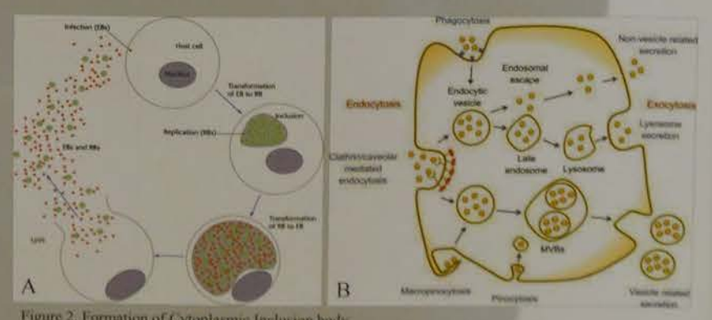


Figure 2. Formation of Cytoplasmic Inclusion body.

Conclusion

Cytoplasmic inclusions, which were identified in 1.83% of the urinary specimens in our hospital, could be used as an indicator of urinary tract infection (UTI) as they were significantly correlated with the other UTI parameters, i.e., OB, LEU and bacteria. In the present case, inclusions were formed from phagocytosis due to UTI. Reoccurrence UTI is one of the risks for CKD which is high prevalence in Taiwan. Urinalysis is a common screening test for many diseases. More information in report, better clinical care we could provide.

Reference

1. Figure 2A: https://www.studyblue.com/notes/note/n/microbiology-urinary-tract-infection/deck/11963921
2. Figure 2B: https://www.researchgate.net/figure/262693063_fig1_Schematic-of-endocytosis-and-exocytosis-patterns-of-nanoparticles-Nanoparticles-enter

Urinalysis

PH-02

Quality Improvement of Image-Based Automatic Urinalysis A Two-PDCA-Cycle Experience

Hui-Szu Tsai, Sheng-Hsiu Wei, Chuan-Po Lee, Ya-Chin Li

Division of Quality Management, Department of Pathology & Laboratory Medicine, Taipei Veterans General Hospital, Taiwan, R.O.C.

Introduction

Automation and auto-verification are trends in laboratories which attempting to get more benefit. Two image-based urine sediment analyzers and auto-verification were introduced to our lab in 2013. In the next two years, we modified the operation procedures and provide more information in the reports. The most important of all, we could be in accordance with national guidelines and even superior than many other laboratories.

PDCA Cycles

In Accordance with National Guidelines

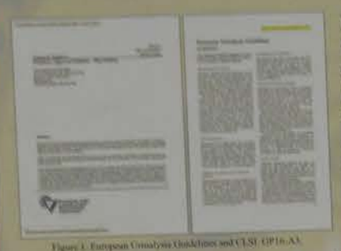


Figure 1. European Urinalysis Guidelines and CLSI GP16-A3.

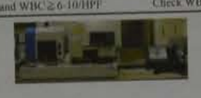
In either CLSI GP16-A3 or European Urinalysis Guidelines, urinalysis should perform in 2 hours after specimen collection. Manual procedures of sediment exam is labor intensive, wide inter-observer variability and time-consuming. With workload of over 200,000 specimens per year, we need a efficient system which could perform good turnaround time (TAT), standardize manual procedures and expand functions. Two image-based automatic analyzers [USCANNER(E)] for sediment examination and auto-verification using 21 rules were introduced to our lab in Nov. 2013.

Application of New System

In February 2014, a total of 4,108 intercepted cases were collected to evaluate the appropriateness of the intercept criteria. Of which only 5.6% (228 reports) were revised after image review. Base on the result, the intercept criteria were modified and reduced to 8 rules in chemistry (strip), image (sediment) and chemistry vs. image logic (Table 1). Nevertheless the renal tubular cell and urothelial cell were not formally reported till Jan. 2015

Table 1. Image-Based Analyzer and Auto-verification Criteria

New Criteria	Check
Chemistry	
OB≧1+	Check RBC
LEU ≧1+	Check WBC
Image	
CAST≧1-2/LPF	Check Cast
CRYSTAL≧0-5/LPF	Check Crystal
Renal Tubular Epithelial Cell≧1/HPF	Check RTE
Urothelial Cell≧1/HPF	Check Uro. Cell
Chemistry vs. Image logic	
OB - and RBC≧ 3-5/HPF	Check RBC
LEU - and WBC≧6-10/HPF	Check WBC



Cycle 1

Improvement of Turnaround Time

There were 10,497, 10,969 specimens in 2013/03 (OPD 7,260, IPD 3,237) and 2014/03 (OPD 7,725, IPD 3,244). Mean TAT of OPD and IPD specimens shortened 7 min and 30 min respectively. The 30 min complete rate of OPD and IPD specimens increased 20% and 57% respectively. Both in OPD and IPD specimens, the 60 min complete rate was over 95% (Table 3). Staff responsible for IPD cases had to operate phlebotomy too, so the new system worked more efficient in IPD specimens. However, ER specimens didn't run in the system yet.

Table 3. Turnaround Time After Applying New System.

Evatulated period		Specimen	Auto-Send (%)	Turnaround Time (Specimen registration to report)						≦30 min (%)	≦60 min (%)
				Mean	SD	Medium	90 percentile	95 percentile	99 percentile		
OPD	2013.03	7,260	**	29.66	12.3	29	44	51	71	57.81	97.35
	2014.03	7,725	68.29	22.57	12.29	20	39	46	62	78.78	98.91
IPD	2013.03	3,237	**	54.4	30.66	48	90	119	144	20.29	64.67
	2014.03	3,244	53.76	22.74	14.93	19	44	52	76.57	77.19	97.04

Efficiency of New System

In March 2014, 3,118 specimens using 8 intercepted rules were collected to evaluate the efficiency (true positive & negative). Of which, 1,641 results were auto-verified and the efficiency was over 95%. Either sensitivity or specificity of RBC, WBC, and cast were over 90% that revealed the system was good for screening simple cases (Table 2).

Table 2. Efficiency of Image-Based Analyzer with Auto-verification.

RBC (>2/HPF)		Microscope		
		POS	NEG	
U-Scan	POS	2	0	100.0% PPV
	NEG	0	1,639	100.0% NPV
		100.0% Sen.	100.0% Spe.	Efficiency 100.00% 1,641

Crystal present		Microscope		
		POS	NEG	
U-Scan	POS	52	0	100.0% PPV
	NEG	13	1,576	99.2% NPV
		80.0% Sen.	100.0% Spe.	Efficiency 99.21% 1,641

RBC/ WBC/Cast		Microscope		
		POS	NEG	
U-Scan	POS	244	0	100.0% PPV
	NEG	16	1,381	98.9% NPV
		93.8% Sen.	100.0% Spe.	Efficiency 99.02% 1,641

WBC (>5/HPF)		Microscope		
		POS	NEG	
U-Scan	POS	242	0	100.0% PPV
	NEG	16	1,383	98.9% NPV
		93.8% Sen.	100.0% Spe.	Efficiency 99.02% 1,641

Cast present		Microscope		
		POS	NEG	
U-Scan	POS	19	0	100.0% PPV
	NEG	0	1,622	100.0% NPV
		100.0% Sen.	100.0% Spe.	Efficiency 100.00% 1,641

RBC/WBC/ Crystal/Cast		Microscope		
		POS	NEG	
U-Scan	POS	296	0	100.0% PPV
	NEG	29	1,316	97.8% NPV
		91.1% Sen.	100.0% Spe.	Efficiency 98.23% 1,641

To Improve the Quality of Reports

Figure 2. More Sediment Particles Introduced in Guidelines.

Important sediment particles:

- Renal tubular cell (RTE)
- Urothelial cell
- Inclusion body
- Oval fat body (OFB)
- Decoy cell
- Isomorphic RBC
- Dysmorphic RBC
- Differential WBC
- Atypical cell

Since Jan. 2015, we commented more sediment particles listed above in reports that was according to guideline announce by Taiwan Society of Laboratory Medicine (TSLM) in the same year. It suggested we could provide more information for clinical care.

Continuous Education and Competent Test

After image-based automatic urinalysis was utilized in 2014, many images captured from instruments or lectures were announced to educate staff (Figure 3 A,B). After one year training, staff were qualified by blind test of 10 specimens then they could verify the reports.

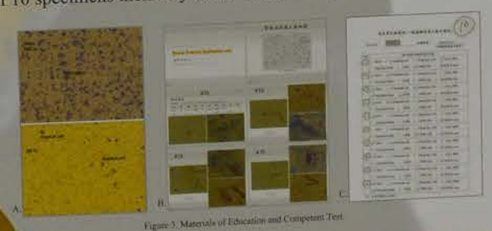


Figure 3. Materials of Education and Competent Test

Cycle 2

Performance of Turnaround Time

Since Jan. 2015, ER specimens were transferred to the system and the mean TAT was 12.8 min with 30 min complete rate 97.8%. Compared with 2014, mean TAT of OPD and IPD specimens shortened 3 and 4 min respectively. The 60 min complete rate of OPD and IPD specimens were 99.4% and 98.5% respectively (Table 6). We performed efficiently and provided more meaningful information for clinical care.

Table 6. Comparison of Turnaround Time in 2014 and 2015

Part	Specimen	Auto-send (%)	TAT (Specimen registration to report)							≦30 min (%)	≦40 min (%)	≦60 min (%)	≦120 min (%)
			Mean	SD	Medium	MAX	90p	95p	99p				
OPD													
2014	135,159	60.8	24.5	15.9	21	159.8	44.1	53.3	80.2	70.1	84.9	96.3	99.7
2015	115,268	54.4	21.7	11.4	20.2	123.4	36.4	41.8	55.4	80.2	93.8	99.4	100
IPD													
2014	64,373	42.3	23.5	16.5	19.2	146.9	43.3	54.2	87.6	76.5	88.5	96.7	99.8
2015	59,941	52.1	19.8	12.8	16.6	130	34.9	43	65.7	85.7	94.1	98.5	99.9
ER													
2014	**	**	**	**	**	**	**	**	**	**	**	**	**
2015	26,526	32.8	12.8	6.9	11.2	74.5	21.8	25.7	35.3	97.8	99.5	100	100

Discovery Rate and Efficiency of Analyzer

RTE had higher discovery rate 8.09% and the sensitivity is 96.38% while the specificity of urothelial cell was better 99.3% in the system (Table 4 & 5).

Table 4. Discovery Rate of different cells

Specimen	OPD	IPD	ER	Total
2015	115,268	59,941	26,526	201,735
Renal Tubular Cell	6.19% (7,131)	9.53% (5,709)	12.59% (3,340)	8.09% (16,330)
Urothelial Cell	2.20% (2,531)	4.23% (2,534)	4.76% (1,264)	3.16% (6,377)
Inclusion body	1.57% (1,813)	1.85% (1,111)	2.78% (737)	1.83% (3,695)
Oval Fat Body	0.34% (393)	0.84% (503	0.74% (196)	0.54% (1,097)

Table 5. Efficiency of RTE and Urothelial Cell Detected by Analyzer.

Renal Tubular Cell		Microscope (Cells Stained)			
		+	-		
USCANNER (TOYOBO)	+	933	2,084	30.92%	PPV
	-	35	8,906	99.61%	NPV
		96.38% Sen.	81.04% Spe.	11,958	

Urothelial Cell		Microscope (Cells Stained)			
		+	-		
USCANNER (TOYOBO)	+	11	81	11.96%	PPV
	-	367	11,499	96.91%	NPV
		2.91% Sen.	99.30% Spe.	11,958	

Discussion

- Limitations of TAT for ER and OPD specimens are 30 min and 60 min. For OPD specimens, we have a internal warning time on 40 min since 2014.
- In 2014, staff were educated to use new analyzer and recognize morphologies of differential cells, so we only had basic items (RBC, WBC, epithelial, crystals, and casts) in the reports. And the ER specimens didn't run in the system till 2015.
- Except the basic items, the software of analyzer can only recognize the RTE and urothelial cell at present. The negative predict value of them are high as 99.6% and 96.9% respectively. The sensitivity of RTE was 96.4% while urothelial cell had better specificity 99.3% (Table 4 & 5). In our experience, the analyzer would mix up RTE with WBC or small cast, so we need confirm by cells stained and observing in microscope.
- The auto-send rate of ER was 32.8% that might due to the complicate specimens from urinary tract infection, kidney stone, chronic kidney disease, and liver diseases. There would have more RBCs, WBCs, renal tubular cells, crystals or casts in the sediment from patients of these diseases.
- We participated in establishing the urine sediment examination guidelines announced by TSLM. So we had one step further than other laboratories in Taiwan in recognizing more particles in urine sediment with experiences from Japanese Association of Medical Technologists (JAMT).

Conclusion

Continuous improvement and education made the staff more competent to face the challenges. And with good automatic analyzers and appropriate intercept criteria for auto-verification, we could perform well as a top laboratory with high efficiency and quality.

Urinalysis
PH-03

Cellulose acetate membrane electrophoresis in the case of a negative or (±) result of the method of urinary protein test paper

Mihoko Nishizawa [1], Ryo Kubota [1], Toru Igarashi [2], Nobue Sakai [1]

1) Graduate Course of Health and Social Services, Saitama Prefectural University
2) Department of Pediatrics, Nippon Medical School Hospital

Introduction

The method of urinary protein test paper has been used as a screening test for kidney disease. There may be an early stage of kidney disease and mild kidney injury even in the case of a negative or (±) result of this assay. We aimed to analyze urinary protein in cases of negative or (±) results on urinary protein test paper using cellulose acetate membrane electrophoresis.

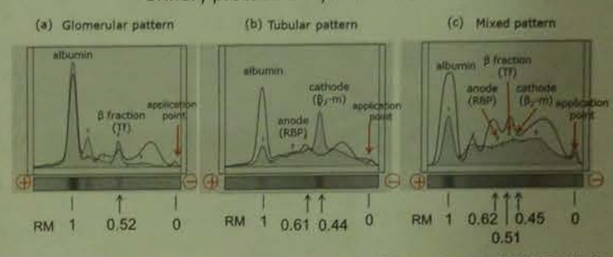
Methods

Urine samples were obtained from 40 patients who were being monitored in the Department of Pediatrics, Nippon Medical School Hospital. The results of analysis of urinary protein test paper in these patients were negative or (±). Analysis of urinary proteins was based on cellulose acetate membrane electrophoresis. After electrophoresis, the proteins were stained with a silver staining solution. The protein fractions from these patients were analyzed in urinary protein analysis software (Fig.1.). This software enables classification of urinary protein patterns into glomerular, tubular, and mixed using relative mobility of each band during cellulose acetate membrane electrophoresis of urine samples from patients.

Results

The glomerular pattern was observed in 9 (22.5%) cases, tubular pattern in 8 (20.0%), mixed pattern in 1 (2.5%), and other patterns in 22 cases (55.0%) (Fig.2., Table 1). This urinary protein analysis software allowed us to identify the type of kidney injury even in patients with a negative or (±) result on urinary protein test paper.

urinary protein analysis software



(Kubota R, et al. J Electrophoresis. 2010;54,13-8.)

Fig.1. Principle of urinary protein analysis software. Urinary protein fractions (gray area) in 3 representative samples ((a) glomerular, (b) tubular, and (c) mixed patterns). The control was serum protein fraction used as a reference (bold line).

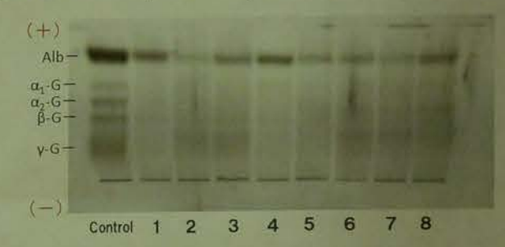


Fig.2. Urinary protein fraction of patients with negative or (±) result of urinary protein test paper using cellulose acetate membrane electrophoresis
No1, 2: glomerular pattern (Negative in test paper)
No3, 4: glomerular pattern ((±) in test paper)
No5, 6: tubular pattern (Negative in test paper)
No7, 8: tubular pattern ((±) in test paper)

Table 1. The type of kidney injury in patients with a negative or (±) result on urinary protein test paper

	glomerular pattern	tubular pattern	mixed pattern	other patterns
N	9 (22.5%)	8 (20.0%)	1 (2.5%)	22 (55.0%)

Conclusions

Analysis of urinary proteins using cellulose acetate membrane electrophoresis may be useful for early detection of damage sites in kidneys.

Urinalysis

PH-04

Classification of uropathogens using Sysmex UF-1000i contributes to empiric therapy of urinary tract infection

Application of bacteria morphology parameters on rapid diagnosis and antibiotic choose prior to urine culture

Ling-Chaio Tsai, Pin-Ching Pan, Chia-hao Chuang , Jin-Biou Chang
...partment of Pathology, National Defense Medical Center, Division of Clinical Pathology, Tri-Service General Hospital, Taipei, Taiwan

Background:

Urinary tract infection (UTI) is a common infection disease in Taiwan. Urine culture is still considered as a gold standard for clinical diagnosis of UTI patients. However, culturing of the samples is both time and labor consuming, with most of samples yielding no growth. Besides, clinicians usually start empiric therapy with antibiotic agents mainly against Gram negative bacteria, but they are not very effective against Gram negative bacteria. A reliable screening tool would be very important for clinicians and laboratories.

Material and methods:

1. Sample collection: We collected 1162 patients submitted to our institutions for suspected UTI during 2016/01/01 to 2016/06/30. Urines were collected separately for urine routine analysis (urine chemistry examination on a ARKRAY AX-4030 analyzer and urine sediment examination on a Sysmex UF-1000i analyzer) and urine microbiological examination.
2. UF-1000i analysis: UTI-info. was provided by Sysmex UF-1000i analyzer by using the criteria of WBC ≧ 10/uL and Bacteria ≧ 100/uL. Beside, bacteria can be distinguished according to the distribution of pathogens on BACT histogram and indicated as "Rods?" or "cocci/mixed?". (Figure 1).
3. Microbiological analysis: The culture was labeled as positive if containing ≧ 10^5 CFU/mL. Standard identification were performed by using Biomeriux Vitek2 analyzer.

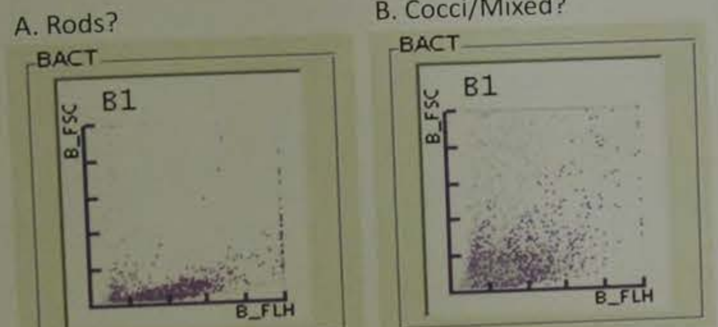


Figure 1. Bacteria morphology were classified as "Rods?"(A) or "Cocci/Mix?"(B) in UTI-suspected samples by using BACT histogram of Sysmex UF-1000i.

Results:

A mono-microbial infection was observed in 449 samples which were recruited for evaluation of bacteria-related information, including UTI-info. and bacteria morphology of Sysmex UF-1000i. Bacterial isolates of this study are reported in Table 1.

	Strains
Achromobacter xylosoxidans	1
Acineto. baumannii	6
B-Streptococcus group B	8
Streptococcus Non-A,B,D	1
...trobacter diversus	6
...itrobacter freundii	3
Coagulase Negative Staphylococcus	2
Diphtheroid	3
Enterobac. aerogenes	2
Enterobacter cloacae	5
Enterococcus faecalis	13
Enterococcus faecium	5
Escherichia coli	291
Gardnerella vaginalis	2
Klebsiella pneumoniae	41
Lactobacillus spp.	5
Morganella morganii	2
Proteus mirabilis	21
Proteus vulgaris	1
Providence rettgeri	1
Providence stuartii	1
Pseudo. aeruginosa	16
Serratia marcescens	5
Staphylococcus aureus	3
Staphylococcus saprophyticus	2
Stenotroph. maltophil	2
Viridans streptococus gr.	1
Total	449

Table 1. Isolated strains from positive (≧ 10^5 CFU/mL) urine samples. 449 samples were observed a mono-microbic infection and recruited in this study.

To determination whether UTI-info. can be used in the screening parameters that would imply a better management of UTI, as well as a high reduction in the workload and cost savings. Screening for negative urine samples requires both a high sensitivity and high NPV in order to minimize the number of false-negative results. The cut-off value was 10 WBC/uL and 100 bacteria/uL, with sensitivity, specificity, positive predicted value (PPV) and negative predictive values (NPV) of 91.8%, 46.8%, 52.1% and 90.0%, respectively (Table 2). It indicated that UTI-info. May be useful as a screening tool for UTI suspected patients.

	Sysmex UF-1000i UTI-info		
Urine culture (>10^5 CFU/mL)	Negative	Positive	Total
Negative	334	379	713
Positive	37	412	449
Total	371	791	1162
Sensitivity	91.8%		
Specificity	46.8%		
PPV	52.1%		
NPV	90.0%		

Table 2. Parameters depending on the cut-off values for UTI-info of Sysmex UF-1000i.

To determine the ability of bacteria morphology identification of Sysmex UF-1000i, we further analyzed 412 UTI-info. and urine culture both positive samples. As reported in Table 3 in presumptive rapid identification of UTI sustained by Rods showed a sensitivity of 70.6%, a specificity of 73.5%, a PPV of 96.7%, a NPV of 18.4%. However, we found that large amount of RBC and WBC in urine samples may interfere bacteria morphology judgment due to obviously debris distribution in bacteria channel of Sysmex UF-1000i analyzer. Therefore, we used a specific rule to transfer the results of RBC 3+ or WBC 3+ samples from "Cocci/Mixed?" to "Rods?". After that, the statically parameters are showed a sensitivity of 93.9%, a specificity of 29.4%, a PPV of 93.7%, a NPV of 30.3%.

	Sysmex UF-1000i Bacteria morphology		
Urine culture (>10^5 CFU/mL)	Cocci/Mixed?	Rods?	Total
Cocci/Mixed?	25	9	34
Rods?	111	267	378
Total	136	276	412
Sensitivity	70.6%		
Specificity	73.5%		
PPV	96.7%		
NPV	18.4%		

Table 3. Parameters depending on the identification of bacteria morphology of Sysmex UF-1000i.

	Sysmex UF-1000i Bacteria morphology		
Urine culture (>10^5 CFU/mL)	Cocci/Mixed?	Rods?	Total
Cocci/Mixed?	10	24	34
Rods?	23	355	378
Total	33	379	412
Sensitivity	93.9%		
Specificity	29.4%		
PPV	93.7%		
NPV	30.3%		

Table 4. Parameters depending on the identification of bacteria morphology of Sysmex UF-1000i combined with a specific rule.

Conclusion: Sysmex UF-1000i analyzer is a reliable screening tool for rule-out of UTI. It also can specifically identify the bacteria with rods morphology (mainly gram negative bacilli) to provide advanced information for empiric therapy before complete of urine culture reports.

The Coexisting of Renal Tubular Epithelial Cells and Cystine Crystals in Acetylcysteine Dysmetabolism Case - A Case Report and Survey

Yen-Li Wang; Chuan-Po Lee; Ya-Chin Li; Hui-Szu Tsai; Hsiu-Chin Fan
Division of General Laboratory, Department of Pathology & Laboratory Medicine, Taipei Veterans General Hospital, Taiwan, R.O.C.

Urinalysis

PH-05

Development of cellulose acetate membrane electrophoresis based urinary proteomics

– Combination of old and new methodology –

Aki Nakayama Howley, Mai Kimino, Kiyoko Shiba, Shiro Iijima
Bunkyo Gakuin University, Tokyo Japan

Abstract

Introduction: Development of non-invasive urine based tests would be a tremendous benefit to patients with renal diseases. A urinary protein panel, which is a renal disease assessment involving several novel protein markers increases the diagnostic accuracy. Cellulose acetate membrane electrophoresis (CAME) coupled with highly sensitive colloidal silver staining is one of the useful methods in analyzing an entire scope of urinary protein abnormalities. In this study, we developed a CAME-based proteome analysis strategy to increase utility urinary protein fraction on CAME.

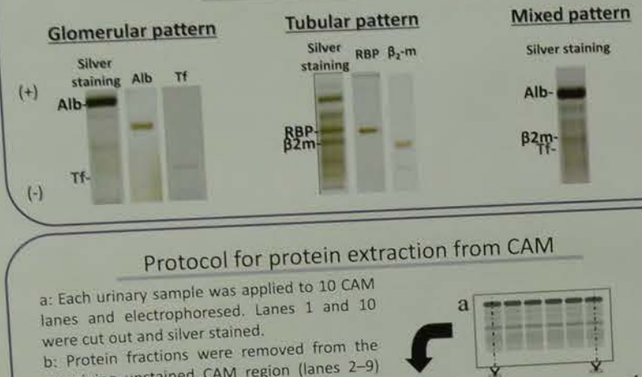
Methods: A urine sample loaded with ten lanes on CAME. After electrophoresis, both ends were cut and stained with colloidal silver. The unstained lane number two to nine were then cut out around the stained area. The membrane strips were further cut into smaller segments and then incubated with a SDS PAGE sample preparation buffer to extract proteins in each fraction. Protein identification was performed by SDS PAGE combined with in-gel digestion and mass spectrometry.

Results: Total 31 proteins were identified in the patients with tubulointerstitial nephritis, including 20 urinary proteins that were newly identified in the present CAME-based proteome analysis. This included beta-2-glycoprotein and alpha-1-B-glycoprotein, candidate markers of exacerbation of renal function.

Conclusion: Combining the conventional method of CAME and the relatively new methodology of proteome analysis enabled us to identify the biomarkers of renal disease. These results increase the utility of urinary protein fraction using CAME as a diagnostic tool in clinical laboratory.

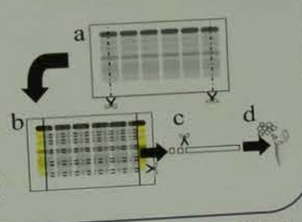
Background

CAM electrophoretic profiles of urinary proteins indicates kidney damage



Protocol for protein extraction from CAM

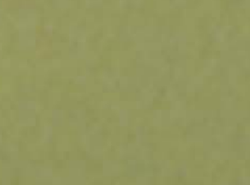
a: Each urinary sample was applied to 10 CAM lanes and electrophoresed. Lanes 1 and 10 were cut out and silver stained.
b: Protein fractions were removed from the remaining unstained CAM region (lanes 2–9) by using the stained membrane as a guide.
c: Each section was further fragmented into 5-mm² sections.
d: The fragments were incubated with 1.5% SDS containing 0.5 M Tris-HCl buffer (pH 6.8), for protein extraction.



Aim: To establish a protein extraction method by using CAM and identify proteins in each fraction by using proteome analysis

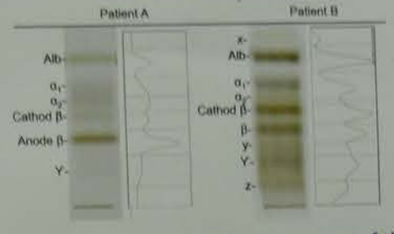
Subjects

	Patient A	Patient B
Gender	Male	Female
Age	57	46
Urinary protein	83.0 mg/L	1313 mg/L
Urinary albumin	12.6 mg/L	160 mg/L
Urinary creatinine	331 mg/L	330 mg/L
Clinical diagnosis	renal insufficiency, sarcoidosis, pneumoconiosis, tubulointerstitial nephritis	HIV (+), drug-induced tubulointerstitial nephritis
Diagnosis by renal biopsy	tubulointerstitial nephritis, minor glomerular abnormality, granuloma	tubulointerstitial nephritis, minor glomerular abnormality.
Medication	–	LPV/RTV, TDF, EFV
Number of protein fractions on CAME	6	9



Results

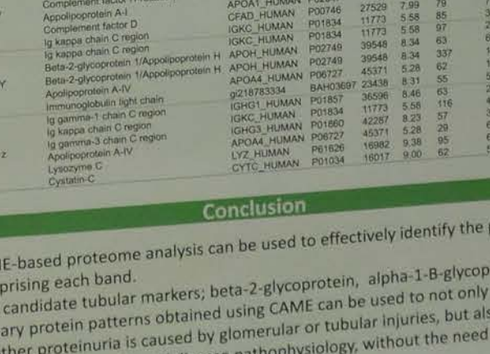
Silver-stained CAM gel and densitometric profiles



Relative mobility of each fraction

Protein fraction	Patient A Fraction (%)	Patient A RM	Patient B Fraction (%)	Patient B RM
x	-	-	2.50	1.14
Alb	13.7	1.00	13.6	1.00
α_1	13.1	0.83	12.3	0.82
α_2	15.3	0.72	5.4	0.73
β_1	12.1	0.62	14.4	0.66
β	-	-	15.4	0.53
β_2	35.6	0.42	-	-
γ	-	-	6.5	0.36
Y	10.3	0.32	19.8	0.32
z	-	-	10.1	0.15

SDS-PAGE patterns of the extracted proteins



Proteins identified by LC-MS/MS

Patient A band no.	Fraction	Protein name	Gene name	AC	Nominal mass	pI value	Score	Peptide #	Sequence coverage
1	Alb	Serum albumin	ALBU_HUMAN	P02768	71317	5.92	104	11	26%
2	α_1	Uromodulin	UROM	P07911	72451	5.05	24	2	3
3		Alpha-1-microglobulin	AMBP_HUMAN	P02760	39886	5.95	136	4	15%
4		Uromodulin	UROM	P07911	72451	5.05	65	4	5%
5	α_2	Zinc-alpha-2-glycoprotein	ZA2G_HUMAN	P25311	34465	5.71	86	6	23%
6		Alpha-1-microglobulin	AMBP_HUMAN	P02760	39886				
7	βa	Retinol-binding protein 4	RET4_HUMAN	P02753	23337	5.76	68	4	30%
8		Serotransferrin	TRFE_HUMAN	P02787	79294	6.81	22	3	7%
9		Prostaglandin-H2 D-isomerase	PTGDS_HUMAN	P41222	21243	7.66	88	2	6%
10	β	Ig kappa chain C region	IGKC_HUMAN	P01834	11773	5.58	31	2	32%
11		Lysozyme C	LYZ_HUMAN	P61626	16982	9.38	79	3	19%
11		Cystatin-C	CYTC_HUMAN	P01034	16017	9.00	176	3	19%
12	Y	Ig gamma-1 chain C region	IGHG1_HUMAN	P01857	36596	8.46	61	3	50%
13		Ig kappa chain C region	IGKC_HUMAN	P01834	11773	5.58	123	3	48%
Patient B									
1	x	Transthyletin	TTHY_HUMAN	P02766	15991	5.52	79	3	17%
2	Alb	Serum albumin	ALBU_HUMAN	P02768	71317	5.92	254	12	48%
3		Alpha-1-antitrypsin	A1AT_HUMAN	P01009	46878	5.37	166	4	10%
4	α_1	Alpha-1B-glycoprotein	A1BG_HUMAN	P04217	54790	5.56	66	9	24%
5		Alpha-1-microglobulin	AMBP_HUMAN	P02760	39886	5.95	76	10	21%
6		Angiotensinogen	ANGT_HUMAN	P01019	53406	5.87	100	3	8%
7		Vitamin D-binding protein	VTDB_HUMAN	P02774	54526	5.40	77	10	32%
8	α_2	Apolipoprotein A-IV	APOA4_HUMAN	P06727	45371	5.28	21	3	13%
9		Zinc-alpha-2-glycoprotein	ZA2G_HUMAN	P25311	34465	5.71	108	4	10%
10		Alpha-1-microglobulin	AMBP_HUMAN	P02760	39886	5.95	130	10	31%
11		Plasma protease C1 inhibitor	IC1_HUMAN	Q96FE0	55347	6.09	89	7	19%
12		Complement C1s subcomponent	C1S_HUMAN	Q9UCV3	78174	4.86	73	9	19%
13		Antithrombin-III	ANT3_HUMAN	Q9UC78	53025	6.32	60	7	21%
14	β_c	Spermatogenesis-associated protein 7	SPAT7_HUMAN	Q9P0W8	68190	5.90	35	3	7%
15		Vitamin D-binding protein	VTDB_HUMAN	P02774	54526	5.40	103	7	28%
16		Apolipoprotein A-IV	APOA4_HUMAN	P06727	45371	5.28	28	3	21%
16		Complement C3 fragment	CO3_HUMAN	P01024	188569	6.02	67	9	10%
17		Retinol-binding protein 4	RET4_HUMAN	P02753	23337	5.76	61	7	45%
18		Apolipoprotein A-IV	APOA4_HUMAN	P06727	45371	5.28	169	8	21%
19	β	Serotransferrin	TRFE_HUMAN	P02787	79294	6.81	148	6	10%
20		Hemopexin	HEMO_HUMAN	P02790	52385	6.55	56	5	16%
21		Beta-2-microglobulin	B2MG_HUMAN	P61769	13715	5.30	34	3	14
22		Pigment epithelium-derived factor	PDEF_HUMAN	P36955	46454	5.97	220	8	20%
23		Beta-2-glycoprotein 1/Apolipoprotein H	APOH_HUMAN	P02749	39548	8.34	55	4	17%
24		Complement factor H-related protein 1	PHR1_HUMAN	Q03591	38766	7.38	37	6	18%
25	γ	Apolipoprotein A-I	APOA1_HUMAN	P02647	30759	5.56	80	9	32%
25		Complement factor D	CFAD_HUMAN	P00746	27529	7.99	79	7	27%
26		Ig kappa chain C region	IGKC_HUMAN	P01834	11773	5.58	85	3	18%
27		Beta-2-glycoprotein 1/Apolipoprotein H	APOH_HUMAN	P02749	39548	8.34	97	2	18%
28		Beta-2-glycoprotein 1/Apolipoprotein H	APOH_HUMAN	P02749	39548	8.34	63	6	28%
29	Y	Apolipoprotein A-IV	APOA4_HUMAN	P06727	45371	5.28	337	15	54%
29		Immunoglobulin light chain	gi218783334	BAH03697	23438	8.31	62	12	29%
30		Ig gamma-1 chain C region	IGHG1_HUMAN	P01857	36596	8.46	55	5	39%
31		Ig kappa chain C region	IGKC_HUMAN	P01834	11773	5.58	63	2	9%
31		Ig gamma-3 chain C region	IGHG3_HUMAN	P01860	42287	8.23	116	4	51%
31	z	Apolipoprotein A-IV	APOA4_HUMAN	P06727	45371	5.28	29	3	9%
32		Lysozyme C	LYZ_HUMAN	P61626	16982	9.38	57	6	18%
33		Cystatin-C	CYTC_HUMAN	P01034	16017	9.00	95	6	37%
							62	5	45%

Conclusion

- CAME-based proteome analysis can be used to effectively identify the proteins comprising each band.
- Two candidate tubular markers; beta-2-glycoprotein, alpha-1-B-glycoprotein
- Urinary protein patterns obtained using CAME can be used to not only determine whether proteinuria is caused by glomerular or tubular injuries, but also improves our understanding of renal disease pathophysiology, without the need for invasive biopsies.

Nakayama A. et al. J Clin Lab Anal. 2015 cla.21863

Urinalysis

PH-06

Urinalysis

PH-06

The Coexisting of Renal Tubular Epithelial Cells and Cystine Crystals in Acetylcysteine Dysmetabolism Case - A Case Report and Survey

Yen-Li Wang; Chuan-Po Lee; Ya-Chin Li; Hui-Szu Tsai; Hsiu-Chin Fan

Division of General Laboratory, Department of Pathology & Laboratory Medicine,
Taipei Veterans General Hospital, Taiwan, R.O.C.

Introduction

An old man came to our emergency room (ER) at 2/22 afternoon due to diarrhea and mild cough with intermittent high fever since 2/20. At ER, tachycardia and fever up to 40℃ was noted. After admission for 4 days, we found a lot of cystine crystals coexisted with renal tubular epithelial cells (RTEs) in urine sediment. Renal tubular epithelial cells have variant morphology, from round to columnar shape. The granules in cytoplasm are magnification. They usually shed from proximal tubule, distal tubule or collecting duct when acute condition, such as acute tubular necrosis, acute interstitial nephritis, acute cellular allograft rejection and acute nephritic or nephritic syndrome. Cystine is a homodimer of the amino acid cysteine. Patients with cystinuria have impairment of renal cystine transport that may decrease reabsorption in proximal tubule and then increase urinary cystine excretion. Additionally, we investigated 1,598 renal tubular epithelial cells present cases in urine sediment and tried to figure the relations between the different parameters of urinalysis.

Case Profile

A 87-year-old male had history of alcoholic liver cirrhosis, irritable bowel syndrome and gastric ulcer. He was ever an alcoholic but quit for 4-5 years. At ER, doctor gave acetaminophen to relieve fever. Lab data showed leukocytosis and elevated segmented neutrophil percentage. Elevated level of CRP (4.05 mg/dl), K (5.2 mmol/l), AST (53 U/l) and total bilirubin (1.79 mg/dl). Urinalysis showed leukocyte esterase 3+, nitrite +, protein 30 mg/dl, occult blood 1+, RBC 6-10/HPF, WBC 3+/HPF, many bacteria and renal tubular epithelial cells presenting. Morganella morganii was isolated from the urine. Under the impression of urinary tract infection (UTI), he was admitted for further care. After admission, Ciproxin 400mg Q12H was given continuously from 2/23 to 3/4 and he had no more fever. He complained urination difficulties, about 150ccx5 times. He refused to have foley catheter initially, so doctor added Harnalidge D (alpha blocker). However, acute urine retention (AUR) was still noted and foley was inserted. Furthermore, his sputum was thick and hard to spit out, acetylcysteine and steam inhalation were used on 2/25. On 2/26, biochemistry data were normal except CRP evaluated (1.95 mg/dl) and total bilirubin (1.69 mg/dl). Many cystine crystals accompanied by renal tubular epithelial cells in urine sediment were reported. Leukocytosis still existed. On 3/4, WBC was normal so that doctor stopped ciprofloxacin treatment. Because the patient wished to remove foley, doctor consulted with the urology experts. Urologist arranged a cystometrogram (CMG) and suggested him to keep urine foley according to the CMG results. After 20 days of admission, the patient accepted to keep foley catheter and was discharged on 3/13 and scheduled with OPD follow up. *(Figure 1 & Table 1)* Cystine crystals are flat colorless plates and have a characteristic hexagonal shape with equal or unequal sides. We can also observe them in polarized light microscope. Moreover, renal tubular epithelial cells are the most significant epithelial cells in urine and can absorb pigments. That's why we could see cystine crystals stick on the cells. *(Figure 2)*

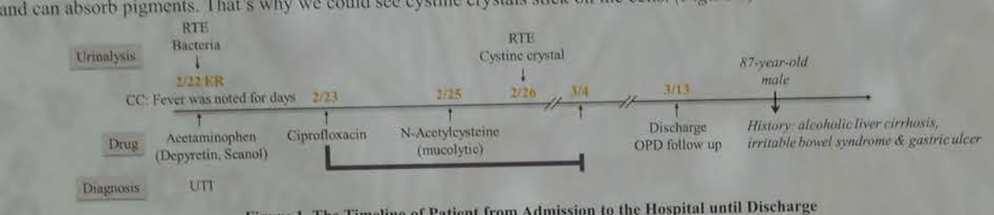


Figure 1. The Timeline of Patient from Admission to the Hospital until Discharge

Table 1. Laboratory Data of Patient

Urine routine	Color	Clarity	GLU (g/dl)	BIL	KET	SG	OB	pH	PRO (mg/dl)	URO (E.U./dl)	NIT	LEU	Sediment
2/22	Dark Yellow	2+	-	-	+/-	1.015	1+	7.5	30	0.1	+	3+	RBC: 6-10/HPF RTE: 0-2/HPF WBC: 3+/HPF Bacteria: many
2/26	Yellow	-	-	-	-	1.010	-	6.5	-	0.1	-	-	RBC: 0-2/HPF RTE: 6-10/HPF WBC: 0-2/HPF Crystal: cystine

Blood culture 2/22	No bacterial growth for 7 days
Urine culture 2/22	Morganella morganii >100000/ML (Ciprofloxacin ≦0.25 S)

CBC, DC	WBC (x10³/μl)	RBC (x10⁶/μl)	Hb (g/dl)	PLT (x10³/μl)	Seg. (%)	Lym. (%)	Mo. (%)	Eo. (%)	Baso. (%)	Imm. Gran. (%)
2/22	11.7	3.69	10.5	221	77.8	8.2	13.6	0.3	0.1	-
2/26	16.1	-	10.0	321	74	6	5	1	1	13
3/4	5.6	-	8.3	139	69.9	17.1	10.2	2.5	0.3	-
Reference	4.5–11	4.6–6.2	14–18	150–350	45–75	20–45	0–9	0–8	0–1	

Chemistry	Na (mmol/l)	K (mmol/l)	Crea (mg/dl)	ALT (U/l)	AST (U/l)	T. Bil (mg/dl)	CRP (mg/dl)	BUN (mg/dl)	eGFR (ml/min/1.73m²)
2/22	134	5.2	1.37	21	53	1.79	4.05	20	49
2/26	135	4.3	0.90	27	-	1.69	1.95	8	80
3/4	139	3.6	0.81	10	-	1.76	-	7	90
Reference	135–147	3.4–4.7	0.7–1.5	0–40	5–45	0.2–1.6	0–0.5	7–20	>60

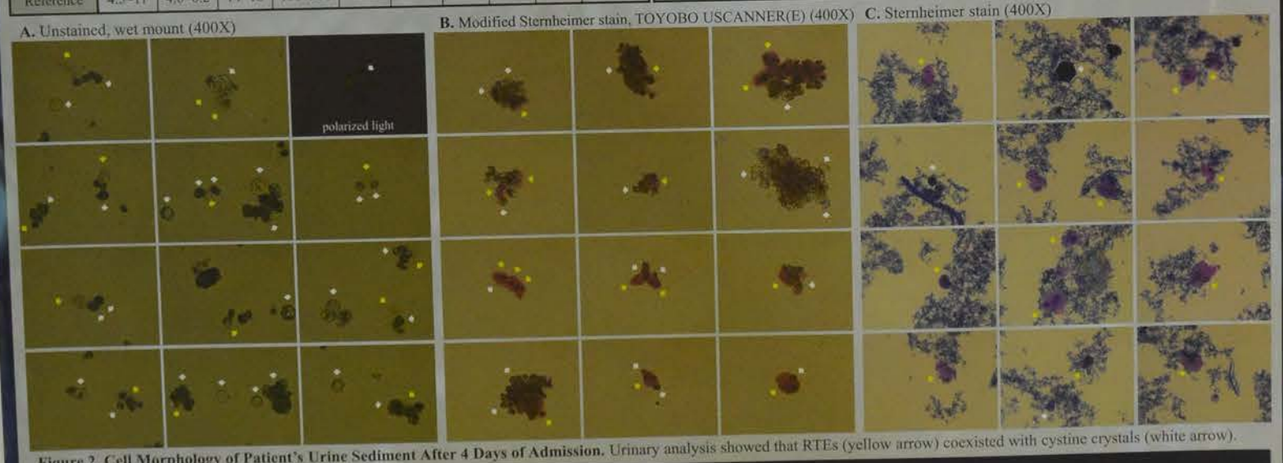


Figure 2. Cell Morphology of Patient's Urine Sediment After 4 Days of Admission. Urinary analysis showed that RTEs (yellow arrow) coexisted with cystine crystals (white arrow).

Survey

There were 1,598 cases of renal tubular epithelial cells in 16,425 urine routine specimens collected during 1 month (2016.02.22~2016.03.22). Since the diseases were more complicated from acute inflammation that patients might be combined two or more syndromes, the discovery rate in ER specimens were the highest (OPD 7.76%, IPD 11.71%, and ER 13.29%). Overall, the discovery rate was 9.73%. *(Table 2)* In 16,425 specimens, we found that when PRO, NIT, LEU of strip were positive and RBC, WBC also existed in sediment, the renal tubular epithelial cells discovery rate were higher. *(Table 3)* On the other hand, the 1,598 renal tubular epithelial cells present cases were also related to OB, PRO, LEU, RBC and WBC highly in urinalysis, over 55% of discovery rate. *(Table 4)*

Table 2. Discovery Rate in Different Departments of Specimens

Departments (2016.02.22~2016.03.22)	OPD	IPD	ER	Total
Specimen volume	9,213	4,782	2,430	16,425
RTE present	715	560	323	1,598
Discovery rate (%)	**7.76%**	**11.71%**	**13.29%**	9.73%

Table 3. Relations of Renal Tubular Epithelial Cells with Urinalysis Results

Parameters (16,425)	GLU	OB	PRO	NIT	LEU
Positive specimen volume	1,985	7,265	5,871	1,196	5,208
RTE present	239	1,048	1,093	284	998
Discovery rate (%)	**12.04%**	**14.43%**	**18.62%**	**23.75%**	**19.12%**
P value (Chi-Square test)	0.0002	<0.0001	<0.0001	<0.0001	<0.0001

Parameters (16,425)	RBC (>2/HPF)	WBC (>5/HPF)	RBC & WBC Co-exist
Positive specimen volume	5,427	3,946	2,493
RTE present	954	898	661
Discovery rate (%)	**17.58%**	**22.76%**	**26.51%**
P value (Chi-Square test)	<0.0001	<0.0001	<0.0001

Table 4. Analysis of 1,598 Renal Tubular Epithelial Cells Present Cases

Parameters (1,598)	GLU	BIL	KET	OB	PRO	URO
RTE present	239	297	425	1,048	1,093	25
Discovery rate (%)	**14.96%**	**18.59%**	**26.60%**	**65.58%**	**68.48%**	**1.56%**

Parameters (1,598)	NIT	LEU	RBC (>2/HPF)	WBC (>5/HPF)	RBC & WBC Co-exist
RTE present	284	998	954	898	661
Discovery rate (%)	**17.77%**	**62.33%**	**59.70%**	**56.20%**	**41.36%**

Discussion

In this case, the patient was treated with acetaminophen and empirical antibiotic (Ciprofloxacin) for antipyretic and bacterial infections, respectively. It is widely known that excessive use of these drugs would cause renal injury. For the subject, the phenomenon reflected immediately at ER. Furthermore, acetylcysteine was given as a mucolytic agent on the third of admission. Urinary cystine crystals occur rarely, usually occur in acidic or neutral urine that are associated with an inherited disorder of metabolism. Cystinuria should be onset during childhood or adolescence. Though stone formation may also occur in infancy and even late adulthood, the stone analysis is important when nephrolithiasis occur. High concentrated cystine in urine may deposit as crystals in proximal renal tubular epithelial cells. As a result, we could observe round eccentric nucleus, granular cytoplasm, and columnar shape which may look like small casts in proximal renal tubular epithelial cells coexisted with cystine crystals in urine sediment. *(Figure 2)* It could be a reference of malfunction in reabsorption. Even general dosage of drugs using in clinical, we should take kidney function into consideration. Renal tubular epithelial cells are pretty sensitive to nephrotoxic drugs and will be impaired after exposure; then shed within 2 hours. In our hospital, there are over 200,000 urinalysis specimens per year. Renal tubular epithelial cells present in urine sediment could be a real-time warning of kidney injury. They not only provide much information for clinical care, but act as an important indicator of early renal damage.

Evaluation of the association between Light's criteria and the microscopic test in pleural effusion

Hizuru Hoshina [1] Chika Miyasaka [2] Miki Hayashi [2] Miho Kasai [2]
Toshiyuki Habara [1] Noriyuki Ozeki [1] Masaru Ozawa [1] Shigeharu Okada [1]

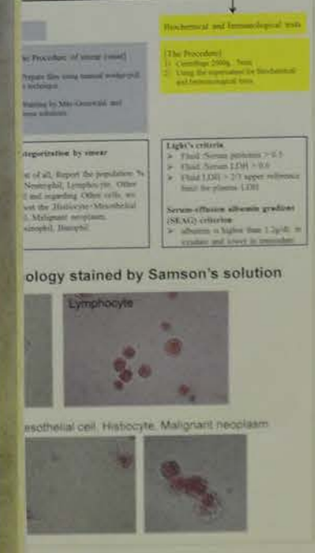
1) Japanese Association of Medical Technologists Committee for standardization of Body Fluid Analysis
2) Suwa Central Hospital

nting and the biochemical measurements are
leural effusion (PE). Light's criteria
otal protein are frequently used for
m transudate, and the discrimination is
nt of the underlying diseases. Our JAMT
dardization for body fluid analysis is
zed method of chamber count and
dy, we compared morphological findings with
ts to consider the significance of cell
rimination of PE.

l the underlying diseases include 78 cardiac
terial inflammation, 65 other conditions from
ntral Hospital (Fig.1). To discriminate exudate
riteria and Serum-effusion albumin gradient
is higher than 1.2g/dL in exudate) were
ical examination. On the other hand, we
differential cell counting as cytological test,
which is higher than 1.0 x 10⁹ cells/L as
ose procedures were written in (Fig.2 and 3)

he specimens

of body fluid analysis



ology stained by Samson's solution

[Results]
252 specimens (72.8%) had the same definition for the biochemic
findings and the cell counting (Table.1). The agreement rate was
lowest in tumor cases and 44.2% specimens of tumor lesions indic
"exudate" for biochemical findings in spite of having poor quanti
leukocytes. Most of them showed increasing of lymphocytic prop
(Fig.4). Some PE from patient with chronic infections contained
1.0 x 10⁹ cells/L cells with also rich lymphocytic proportion, ever
biochemical findings indicated transudate. And, in the other of
discrepancies (biochemical and cytological criteria indicated exu
and transudate, respectively) of the bacterial infection cases,
tuberculosis and chronic inflammation tended to be dominated by
lymphocytes, and the recurrences of pneumonia or pyothorax sho
increasing of neutrophils population.

Table.1: The agreement and discrepancy rate in both crite

Diseases			Biochemical Criteria* Exudate	Biochemical Criteria* Transudate	Agreement	Discre
Heart failure (n=78)	Cytological Criteria**	Exudate	13	3	83	
		Transudate	12	50	80.8%	19
Tumor (n=98)		Exudate	40	2	46	4
		Transudate	38	9	53.5%	46
Bacterial inflammation (n=117)		Exudate	73	5	92	2
		Transudate	20	19	78.8%	21
Other conditions (n=65)		Exudate	24	3	91	5
		Transudate	13	27	78.5%	21

* Light's criteria and SEAG criterion
** Nucleated cell count is higher than 1.0 x 10⁹ cells/L

Fig.4: The differential results in discrepant specimens which determined as exudate by biochemical and transudate by cytological finding

[Conclusion]
The result of the biochemical and the cytological criteria to distingu
exudate from transudate had high agreement rate in PE. While, we
found that the discrepancies in the tumor and the other cases were
dominated by lymphocytes. It is important for diseases treatment to
recognize the feature of body fluid. The differentiation of all nucleat
cells including leukocytes and other cells (e.g. tumor, mesothelial cel
and so on) can help to characterize more accurately in difficult cases
define "exudate" or "transudate" with only Light's criteria and the
number of white cells.
In addition, " The three categorization" are convenient methods whic
is recently advocated by Japanese Association of Medical Technologi
regarding the classification method by chamber counting

[Conflict of Interest (COI) of the Principal Presenter]
No potential COI to disclose

[Contact]
Tel +81 0266 72 1800
kensa@suwachuo.jp

Urinalysis

PH-07

Influence of Vitamin C on Urine Dipstick Test Results

Kyoung A Kim and Yong Lim*

Haedong Hospital, Busan, Korea

**Department of Clinical Laboratory Science, Dong-eui University, Busan, Korea*

Abstract

Vitamin C is a strong reducing agent found at high levels in various foods, and it may influence the results of urine strip tests even at an ordinary consumption levels. After oral administration, we measured urine vitamin C levels using urine strips and evaluated whether vitamin C interfered with various test items. The utility of a urine strip with a vitamin C indicator was assessed. Twenty-five healthy volunteers each ingested 1,000 mg of vitamin C. Their urine samples were tested for vitamin C using a URiSCAN 11 strip (YD Diagnostics, Korea) before and after administration of vitamin C. Standard materials were added to normal pooled urine to generate urine samples with various concentrations of the analytes tested (blood, bilirubin, nitrite, leukocytes, and glucose), and vitamin C was spiked to predetermined levels. These samples were then tested using two urine strips – URiSCAN and Chemstrip test strip (Roche Diagnostics, Germany) - to evaluate interference from vitamin C. In clinical samples with positive vitamin C results, microscopic and chemical analyses were also conducted to examine the differences. Nine urine samples from the 25 volunteers were positive for vitamin C before ingestion, and all subjects were positive after ingestion. Vitamin C spiking of urine demonstrated false-negative results at various concentrations. Vitamin C in urine can cause significant interference with urine strip tests. A urine strip with a vitamin C indicator is useful to reduce the risk of incorrect results in regard to disease states.

Introduction

Vitamin C is a strong reducing agent present in many foods. Its antioxidant properties are thought to help prevent cardiovascular disease and aging. For that reason, the consumption of vitamin C has become popular. Vitamin C is also involved in cancer prevention and improving the quality of life of cancer patients. Urinalysis (UA) is noninvasive, simple, and one of the most important screening tests in clinical practice. UA provides information that can lead to the early detection of diseases in many organs. Urine strip products are available from many diagnostic companies worldwide, and common test items include blood, bilirubin, urobilinogen, ketones, protein, nitrite, glucose, pH, specific gravity (SG), and leukocytes. UA strips are coated with reagents for the measurement of each analyte, and chemical reactions produce color gradations to be measured semi-quantitatively. Many of these reactions are oxidation reactions, and the degree of oxidation is proportional to the concentration of the analyte tested. The presence of vitamin C, an antioxidant, in urine may therefore cause false-negative results for some test items. This is especially problematic for blood and glucose, which are detected via the peroxidase reaction. False-negative results may also occur for nitrite, bilirubin, and leukocytes. Many diagnostic companies have sought to develop UA strips resistant to such interference. Alternatively, one company (YD Diagnostics, Korea) has launched a UA strip with a vitamin C test to additionally measure the vitamin C concentration in urine. This study had two objectives: to evaluate the utility of this vitamin C strip by testing urine samples after vitamin C ingestion and to investigate the effects of vitamin C on other test results.

Results

Table 1. Results using the vitamin C stick, before and after consumption of a vitamin C supplement.

After/Before	Negative	1+	2+	3+	Total
Negative	0	1	12	7	20
1+	0	0	3	8	11
2+	0	0	0	0	0
3+	0	0	0	2	2
Total	0	1	15	17	33

Table 2. Interference by ascorbic acid using the URiSCAN 11 strip.

Titrated concentration		Ascorbic acid (mg/dL) 0	10	50	100	500
Glucose (mg/dL.)	0	Neg.	Neg.	Neg.	Neg.	Neg.
	100	±	±	±	±	Neg.
	250	2+	1+	1+	1+	Neg.
	500	3+	3+	2+	3+	Neg.
	2000	4+	4+	4+	4+	3+
Blood (RBC/μL.)	0	Neg.	Neg.	Neg.	Neg.	Neg.
	5	±	±	±	±	Neg.
	10	1+	1+	1+	2+	1+
	250	3+	3+	3+	3+	3+
Leucocytes (WBC/ μL)	0	Neg.	Neg.	Neg.	Neg.	Neg.
	10	±	±	Neg.	Neg.	Neg.
	25	1+	1+	Neg.	Neg.	Neg.
	500	3+	3+	Neg.	Neg.	Neg.
Nitrite (mg/dL.)	0	Neg.	Neg.	Neg.	Neg.	Neg.
	0.05	Pos.	Pos.	Neg.	Neg.	Neg.
Bilirubin (mg/dL.)	0	Neg.	Neg.	Neg.	Neg.	Neg.
	0.5	1+	1+	Neg.	Neg.	Neg.
	1	2+	2+	2+	1+	Neg.
	3	3+	3+	3+	2+	1+

Table 3. Interference by ascorbic acid using the Chemstrip.

Titrated concentration		Ascorbic acid (mg/dL) 0	10	50	100	500
Glucose (mg/dL.)	0	Neg.	Neg.	Neg.	Neg.	3+
	100	±	1+	1+	1+	3+
	250	2+	2+	2+	2+	3+
	500	3+	3+	3+	3+	3+
	2000	3+	3+	3+	3+	3+
Blood (RBC/μL.)	0	Neg.	Neg.	Neg.	Neg.	4+
	5	±	1+	±	±	4+
	10	1+	1+	1+	2+	4+
	250	4+	4+	4+	4+	4+
Leucocytes (WBC/ μL)	0	Neg.	Neg.	Neg.	Neg.	Neg.
	10	±	±	Neg.	Neg.	Neg.
	25	1+	1+	Neg.	Neg.	Neg.
	500	2+	2+	Neg.	Neg.	Neg.
Nitrite (mg/dL.)	0	Neg.	Neg.	Neg.	Neg.	Neg.
	0.05	Pos.	Neg.	Neg.	Neg.	Neg.
Bilirubin (mg/dL.)	0	Neg.	Neg.	Neg.	Neg.	Neg.
	0.5	Neg.	Neg.	Neg.	Neg.	Neg.
	1	±	±	±	1+	±
	3	2+	2+	1+	±	Neg.

Table 4. Discrepant results due to the presence of vitamin C between the urine strip test and confirmatory reference methods.

Specimen number	Test	Urine strip results	Reference method results	Vitamin C concentration
1	Blood	Negative	3-5 RBC/HPF	2+
2		Negative	3-5 RBC/HPF	2+
3		Negative	6-10 RBC/HPF	3+
4		Negative	3-5 RBC/HPF	3+
5		Negative	3-4 RBC/HPF	3+
6	Leukocytes	Negative	3-5 WBC/HPF	1+
7		Negative	3-5 WBC/HPF	1+
8		Negative	3-5 WBC/HPF	2+
9		Negative	3-5 WBC/HPF	2+
10		Negative	3-5 WBC/HPF	2+
11		Negative	6-10 WBC/HPF	2+
12		Negative	3-5 WBC/HPF	3+
13		Negative	6-10 WBC/HPF	3+
14	Glucose	Negative	122.5 mg/dL	3+

HPF: high power field.

Conclusion

The proportion of samples with false negative results due to vitamin C was small. However, when viewing an individual test with all 'normal' results and positive vitamin C, false negatives due to vitamin C cannot be ruled out. Thus the vitamin C strip may help physicians to more appropriately interpret UA results. To reduce costs, 'normal' UA test results are not usually followed up with additional confirmatory tests. However, the results of this study show that overlooking the presence of vitamin C in urine may lead to potentially serious false-negative results, especially for glucose and blood. Thus, a vitamin C-resistant strip or a strip with a vitamin C indicator is a preferred solution. For specimens positive for vitamin C, the results should be reported with a comment, such as "Vitamin C in urine can cause false-negative results for glucose and blood cells. Repeating a test after restriction of vitamin C consumption is recommended," or the specimens should be subjected to additional follow-up analyses. Vitamin C-resistant strips do not eliminate possible interference by high concentrations of vitamin C; thus, using a strip with a vitamin C indicator is preferable.

Urinalysis

PH-08

Evaluation of the association between Light's criteria and the microscopic test in pleural effusion

Hizuru Hoshina [1] Chika Miyasaka [2] Miki Hayashi [2] Miho Kasai [2]
Toshiyuki Habara [1] Noriyuki Ozeki [1] Masaru Ozawa [1] Shigeharu Okada [1]

1) Japanese Association of Medical Technologists Committee for standardization of Body Fluid Analysis
2) Suwa Central Hospital

[Introduction]
The microscopic cell counting and the biochemical measurements are performed to estimate a pleural effusion (PE). Light's criteria determined by LDH and total protein are frequently used for distinguishing exudate from transudate, and the discrimination is significant for the treatment of the underlying diseases. Our JAMT working group of the standardization for body fluid analysis is considering the standardized method of chamber count and differentiation. In this study, we compared morphological findings with biochemical measurements to consider the significance of cell differentiation in the discrimination of PE.

[Methods]
We examined 346 PE and the underlying diseases include 78 cardiac failure, 86 tumor, 117 bacterial inflammation, 65 other conditions from 2013 to 2016 in Suwa Central Hospital (Fig.1). To discriminate exudate from transudate, Light's criteria and Serum-effusion albumin gradient (SEAG criterion; albumin is higher than 1.2g/dL in exudate) were demonstrated as biochemical examination. On the other hand, we examined numerical and differential cell counting as cytological test, and defined the specimen which is higher than 1.0×10^9 cells/L as exudate. The details of those procedures were written in (Fig.2 and 3)

Fig.1: The details of the specimens

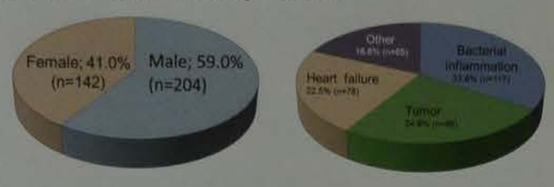


Fig.2: The procedure of body fluid analysis

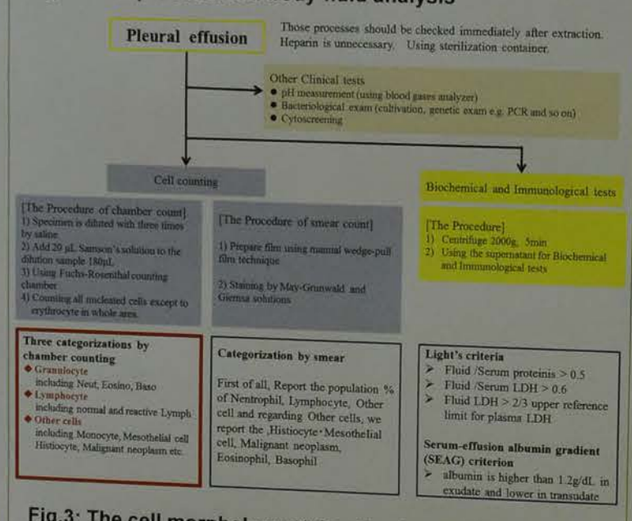
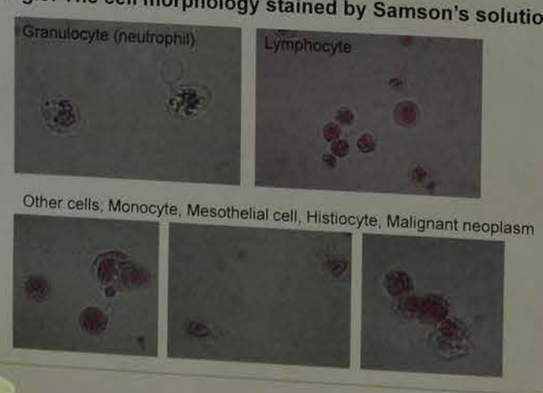


Fig.3: The cell morphology stained by Samson's solution



[Results]
252 specimens (72.8%) had the same definition for the biochemical findings and the cell counting (Table.1). The agreement rate was the lowest in tumor cases and 44.2% specimens of tumor lesions indicated "exudate" for biochemical findings in spite of having poor quantity of leukocytes. Most of them showed increasing of lymphocytic proportion (Fig.4). Some PE from patient with chronic infections contained over 1.0×10^9 cells/L cells with also rich lymphocytic proportion, even biochemical findings indicated transudate. And, in the other of discrepancies (biochemical and cytological criteria indicated exudate and transudate, respectively) of the bacterial infection cases, tuberculosis and chronic inflammation tended to be dominated by lymphocytes, and the recurrences of pneumonia or pyothorax showed increasing of neutrophils population.

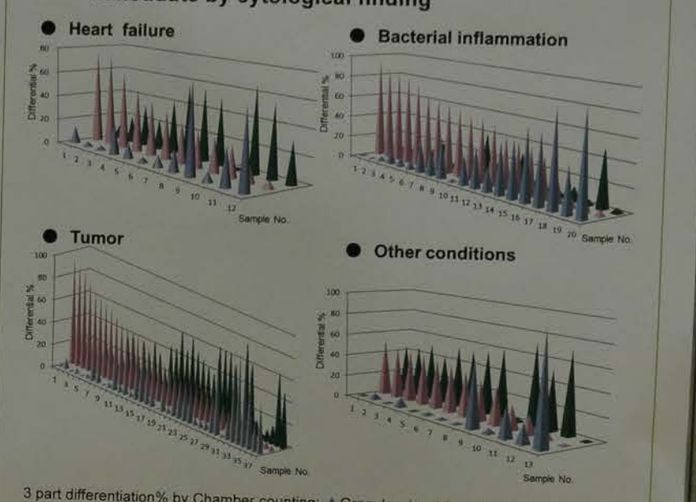
Table.1: The agreement and discrepancy rate in both criteria

Diseases		Biochemical Criteria*		Agreement	Discrepancy
		Exudate	Transudate		
Heart failure (n=78)	Exudate	13	3	63	15
	Transudate	12	50	80.8%	19.2%
Tumor (n=86)	Exudate	40	2	46	40
	Transudate	38	6	53.5%	46.5%
Bacterial inflammation (n=117)	Exudate	73	5	92	25
	Transudate	20	19	78.6%	21.4%
Other conditions (n=65)	Exudate	24	1	51	14
	Transudate	13	27	78.5%	21.5%

Cytological Criteria**

* Light's criteria and SEAG criterion
** Nucleated cell count is higher than 1.0×10^9 cells/L

Fig.4: The differential results in discrepant specimens which determined as exudate by biochemical and transudate by cytological finding



3 part differentiation% by Chamber counting; ▲Granulocyte, ▲Lymphocyte, ▲Other cell

[Conclusion]
The result of the biochemical and the cytological criteria to distinguish exudate from transudate had high agreement rate in PE. While, we also found that the discrepancies in the tumor and the other cases were dominated by lymphocytes. It is important for diseases treatment to recognize the feature of body fluid. The differentiation of all nucleated cells including leukocytes and other cells (e.g. tumor, mesothelial cells and so on) can help to characterize more accurately in difficult cases to define "exudate" or "transudate" with only Light's criteria and the number of white cells.
In addition, " The three categorization" are convenient methods which is recently advocated by Japanese Association of Medical Technologists, regarding the classification method by chamber counting

[Conflict of Interest (COI) of the Principal Presenter]
No potential COI to disclose

[Contact]
Tel: +81 0266 72 1000
kensa@suwachuo.jp

Urinalysis

PH-09

Analysis of urinary protein components in individuals with orthostatic proteinuria

Ryo Kubota [1)], Mihoko Nishizawa [1)], Toru Igarashi [2)], Kiyoko Kanamori [3)], Kiyoko Shiba [3)], Nobue Sakai [1)]
1) Graduate Course of Health and Social Services, Saitama Prefectural University,
2) Department of Pediatrics, Nippon Medical School Hospital,
3) Faculty of Health Science Technology, Bunkyo Gakuin University

Introduction

Orthostatic proteinuria is diagnosed through a lordotic load test. When only orthostatic proteinuria is present, the disorder is thought to be benign with benign causes that will likely disappear over time. We suggested that close observation is necessary in such cases because the proteins excreted in the urine of individuals with orthostatic proteinuria are the same as those excreted in the urine of individuals who have proteinuria with renal disease. Therefore, we analyzed the urinary protein components after the lordotic load test to detect specific urinary proteins, in individuals with orthostatic proteinuria.

Subjects

Urine samples before and after lordotic load testing for 5 min were obtained. The samples yielded negative results for urinary proteins before lordotic load testing.

The thical committee of Saitama Prefectural University approved this study, and informed consent was obtained from all the subjects.

Methods

The upright lordotic posture was produced by placing a bar (4 cm in diameter, 100 cm in length, and 411 g in weight) on the lumbar spine for 5 min. The bar was held in the arms of the subjects such that it was parallel to the ground. The upper part of the body was curved at a 20-degree angle from the waist.

We collected urine samples before the lordotic load test. After the subjects were given 180 ml of water, they assumed the upright lordotic position for 5 min. Urine was collected at 30 min after lordotic load testing.

The urine samples after the lordotic load test were analyzed using cellulose acetate membrane electrophoresis, SDS-PAGE, and 2D electrophoresis.

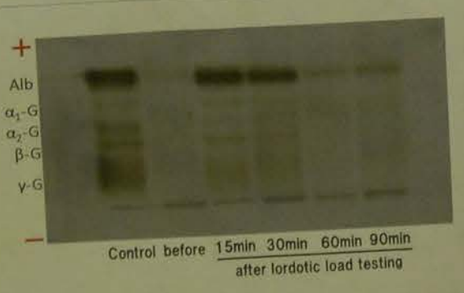


Fig. 1. Urinary protein fractions by cellulose acetate membrane electrophoresis in follow-up after lordotic load testing.

Results

When the urine samples from before the lordotic load test and 30, 60, and 90 min after the lordotic load test were analyzed using cellulose acetate membrane electrophoresis, the urine sample from 30 min after the lordotic load test showed a similar pattern to the serum protein fraction. However, the urine samples from 60 and 90 min after the lordotic load test showed protein patterns similar to those observed before the lordotic load test (Fig.1.).

Urine samples after the lordotic load test showed specific bands at 27.7 and 39.2 kDa, based on the SDS-PAGE results (Fig.2.). When the specific bands at 27.7 kDa and 39.2 kDa were analyzed by 2D electrophoresis, spots for apolipoprotein A1 at 27.7 kDa and haptoglobin at 39.2 kDa were detected using the SWISS-2D PAGE database (Fig.3.).

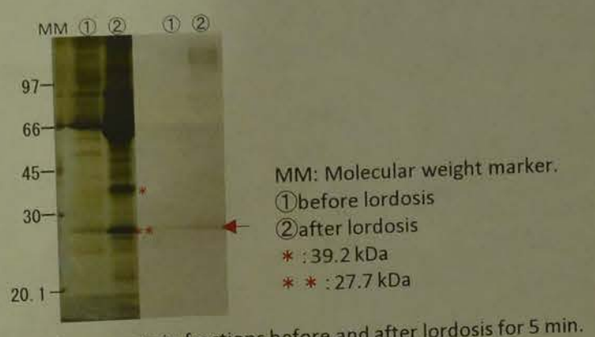


Fig. 2. Urinary protein fractions before and after lordosis for 5 min. (Left)
Detection of various apolipoprotein A1 using western blotting. (Right)

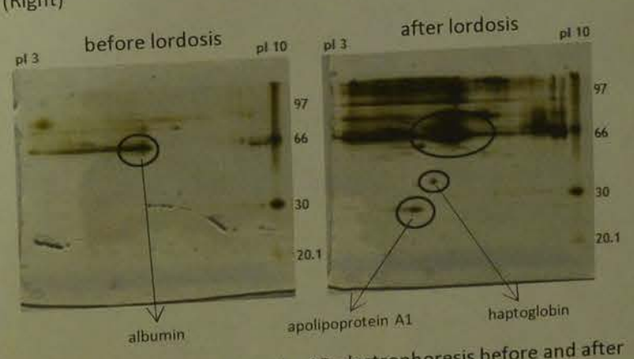


Fig. 3. Urinary protein spots by 2D electrophoresis before and after lordosis for 5 min.

Conclusions

The detection of urinary apolipoprotein A1 and haptoglobin may be useful for diagnosing orthostatic proteinuria.

Urinalysis

PH-10

The exam
of the formula (Tan

Emi OBAYASHI, Yoshiaki UCHIDA, Kaori ABE, Kaz
Iwaki Prefectural Central Hospital, Iwaki, Japan

Introduction

Materials and Methods

Results

Conclusion

Laboratory Information System

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Norihiko AM
1) Department
2) Department
University of

Urinalysis

PH-10

The examination for outpatients of the formula (Tanaka-formula)

Erina KOBAYASHI, Yoshiaki UCHIDA, Kaori ABE, Nanae TAKANO

Ibaraki Prefectural Central Hospital, Ibaraki, Japan

Introduction

Stroke, high blood pressure, and heart disease can be attributed to high salt intake. Tanaka et al. developed a formula (T-formula) that uses the sodium and creatinine concentrations in spot urine specimens to estimate salt intake. However, the formula is not accurate enough for clinical use.

In this study, to improve accuracy of the T-formula, we tested different factors that could cause a discrepancy between estimated and measured sodium intake and can affect the formula's accuracy.

Materials and Methods

1. Materials

269 spot urine samples used in this study were collected June 15th to July 28td in 2015 in Ibaraki Prefectural Central Hospital.

Characteristics Male : N=154, Female : N=115 Age(years):65±17

2. The estimated formula (T-formula): $21.98 \times \{\{SUNa(mEq/L)/SUCr(mg/L)\} \times -2.04 \times age + 14.89 \times weight(kg) + 16.14 \times height\ (cm) - 2244.45\} 0.392 \times 0.0585$

SUNa=Na concentration in the spot voiding urine

SUCr=creatinine concentration in the spot voiding urine

3. Estimation of the T-formula was assessed by the correlation between the electronic record values of infusion and medicine and the values calculated by the T-formula in patients going without a meal.
4. Examination method of salt intake.

Examined on the patients' dietary intake recorded electronically for 7 days from taking samples.

5. Focused on 13 independent factors

· Gender	· GFR<60mL/minute	· High sodium included infusion
· Hypertensives	· GFR<90mL/minute	· Diuretic medicine
· BMI	· Urine sugar	· High sodium included medicine
· Obesity	· Urine protein	· Thin · Infusion

6. Statistical analysis: Pearson's correlation coefficients and Mann-Whitney U test

Results

Table1. Correlation analysis between infusion/ high sodium included medicine and estimated value from T-formula

Day before	Correlation value (r)
1	0.78
2	0.75
3	0.66
4	0.69
5	0.68
6	0.61
7	0.61

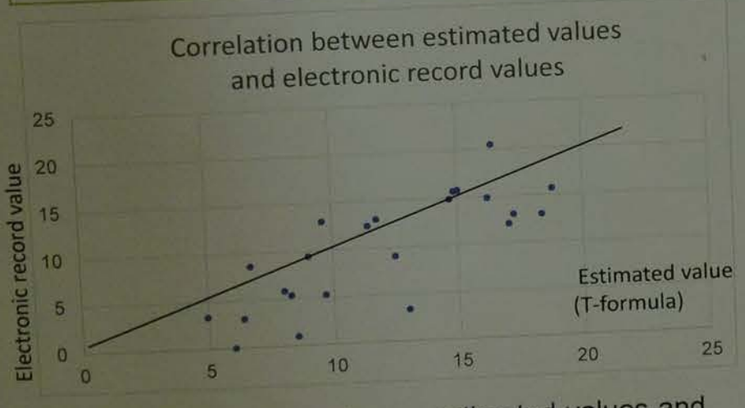


Fig 1. Correlation between estimated values and electronic record values of infusion and medicine in patients going without a meal (1days before).

T-formula values was shown overestimate salt intake but there was significantly correlation between these two factors.

Table2. A discrepancy between estimated and measured sodium intake.

Day before	Median value (g/day)
1	4.1
2	4.1
3	4.03
4	4.17
5	4.34
6	4.24
7	4.38

Table3. Inhibition factor of T-formula

Factor	P-value
Thin	<0.01
Infusion	<0.01
High sodium included infusion	<0.01
High sodium included medicine	<0.01
BMI	<0.01
Diuretic medicine	0.02
Obesity	0.02
Urine sugar	0.02
Hypertensives	0.03
Gender	0.03
Urine protein	0.04
GFR<90mL/minute	0.38
GFR<60mL/minute	0.92

Conclusion

✓ This study shows the value of T-formula because the estimated value from the formula and the real intake of salt have some relation each other (Table1,Fig1).

✓ The T-formula can estimate the intake of salt 3days before if you care the urine sugar, the urine protein, the hypertension, the thin and the high sodium from infusion or medicine (Table 2,3).

✓The high sodium intake from infusion or medicine doesn't need to consider because the outpatients seldom use them.

✓The error of the formula can be related with the Na absorption by the disfunction of renal tubule involved with the urine sugar or the urine protein.

✓ If the patient is thin, they have less muscles. So the 24h-Urine Creatinine of the thin patient differs from the normal one, it may cause some kinds of error in the formula.

✓The formula is very useful when we realize it tends to overestimate the salt intake.

Urinalysis

PH-11

Is urinary hyaline cast a new biomarker for CKD with non-albuminuria stage?

Masato Hoshi[1, 2], Mariko Ishida[3], Hidekazu Ishida[3], Seiko Ushimaru[3], Isao Inagaki[2], Nobuyuki Furuta[3], Hiroyasu Ito[2] and Mitsuru Seishima[2]

1) Department of Biomedical and Analytical Sciences, Fujita Health University of Health Sciences
2) Department of Informative Clinical Medicine, Gifu University Graduate School of Medicine
3) Department of Clinical Laboratory, Gifu University Hospital

Introduction

Chronic kidney disease (CKD) significantly contributes to the increased number of dialysis patients with end stage renal disease. A Japanese CKD risk classification established in 2012, which is defined by albuminuria, eGFR values and underlying disease, demonstrates the relative risks of CKD in great detail. Although CKD with albuminuria stage can be detected by using urine test paper, the screening test for CKD with non-albuminuria stage is not established. In this study, we evaluated the clinical significance of urinary hyaline cast (HC) as a biomarker in CKD with non-albuminuria stage.

GFR stages	GFR (mL/min/1.73m²)	Urine Protein −~±	Urine Protein ≧1+
G1	≧90	28.03	0.61(0.6%)
G2	60~89	61.87	1.71(1.7%)
G3a	45~59	8.86(8.6%)	0.58(0.6%)
G3b	30~44	1.06(1.0%)	0.24(0.2%)
G4	15~29	0.10(0.1%)	0.09(0.1%)
G5	<15	0.01(0.01%)	0.04(0.03%)

Table.1 The number of CKD patients in Japan (×million people)

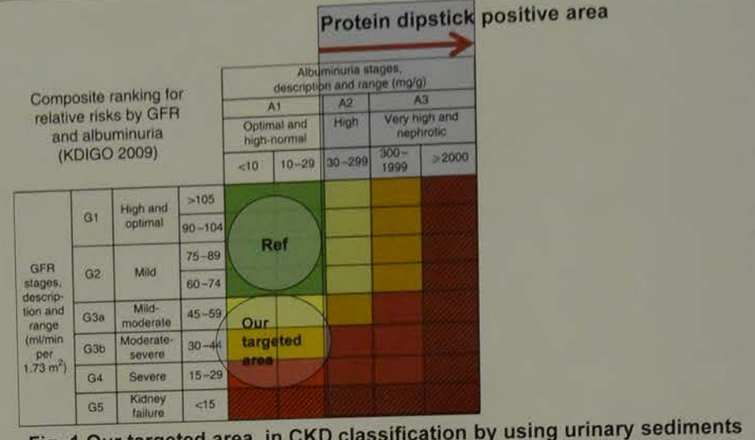


Fig. 1 Our targeted area in CKD classification by using urinary sediments

Materials and Methods

We have categorized 187 non-albuminuria patients into 2 groups (reference and CKD), and we have calculated ROC curves, AUC of the ROC, sensitivity, specificity for diagnosis of CKD, as well as odds ratios by using various kidney function markers, including urinary sediment. Further, we have demonstrated the relationship between the number of HC and various kidney function markers.

Results

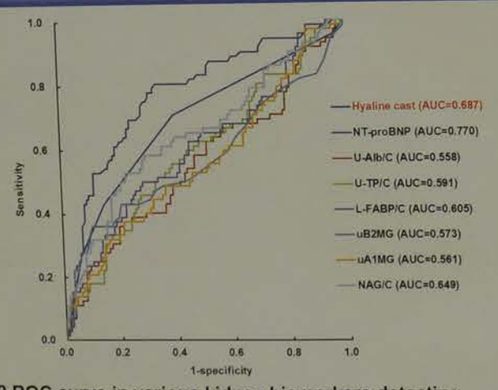


Fig.2 ROC curve in various kidney biomarkers detecting CKD with non-albuminuria stage

	Cut-off	Sensitivity	Specificity	Odds ratio
Hyaline cast	10-29	70.8%	60.0%	3.64
NTproBNP	55	80.6%	66.1%	8.91
U-ALB	10.28	36.1%	80.0%	–
U-TP	0.07	45.8%	72.2%	–
L-FABP	2.57	50.0%	71.3%	6.02
uB2MG	0.270	36.1%	89.6%	4.44
uA1MG	6.7	37.5%	78.3%	2.53
NAG	7.2	52.8%	77.4%	2.59

Table.2 Cut-off value in various kidney biomarkers detecting CKD with non-albuminuria stage

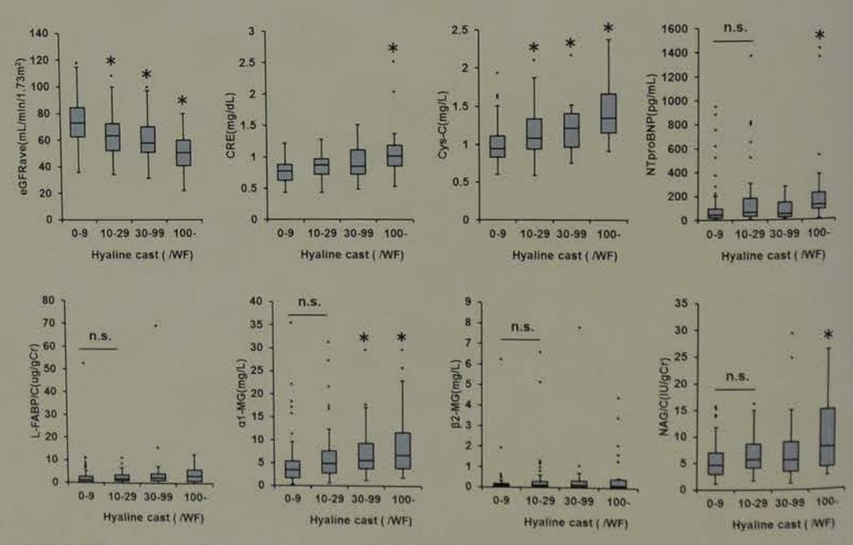


Fig.3 Comparison between the number of hyaline cast and various biomarker in CKD with non-albuminuria stage

Discussion

Our present study suggests that the presence of >10 HC /WF indicates decreased eGFR, and the HC counting may be important and useful for the screening and early detection of CKD with non-albuminuria stage.

FUJITA HEALTH UNIVERSITY
School of Health Sciences

A-2

Urinalysis
PH-12

Fabry disease can be found by urinary sediments

Masaki Hotta[1], Wataru Kobayashi[1], Aya Iwata[1], Kaori Doi[1], Ikuhiro Maeda[1], Toru Takano[2], Yoh Hidaka[2], Norio Sakai[3]

Department of Medical Technology, Osaka University Hospital[1],
Laboratory for Clinical Investigation, Osaka University Hospital[2],
Division of Health Sciences, Osaka University Graduate School of Medicine[3]

Introduction

Fabry disease is an X-linked inborn error in metabolism characterized by the lack of lysosomal hydrolase α-galactosidase A activity. Its pathological feature is the accumulation of globotriaosylceramide (GL-3) in lysosomes, particularly in the vascular endothelium of the kidney, heart and brain. It is a relatively common disease among the lysosomal diseases. Since it can be found in varied age groups and it presents various symptoms, several departments such as pediatrics, internal medicine, cardiology, neurology, dermatology, otolaryngology, and ophthalmology should be engaged in its diagnosis. Screening of Fabry disease is difficult and time-consuming, since its diagnosis usually accompanies same specialized examinations.

Recently, some studies reported that the detecting mulberry bodies in urinary sediments of the patients of Fabry disease is useful in diagnosis. We investigation the result of mulberry bodies which is useful marker of Fabry disease's patient almostly.

Mulberry body

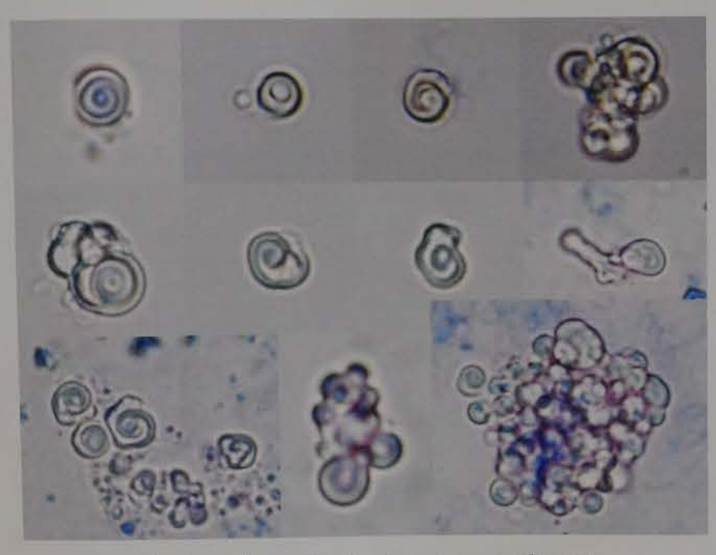


Fig.1 mulberry bodies in urinary sediments

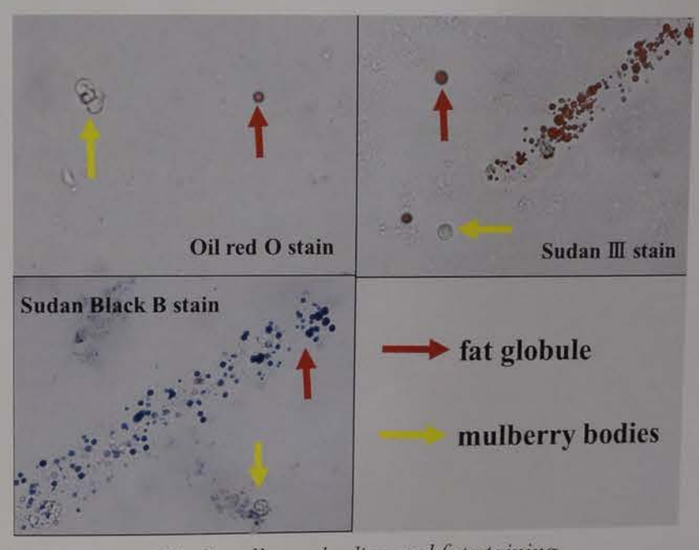


Fig.2 mulberry bodies and fat staining

- Majority of mulberry bodies are in the size around 5～15μm and transparent.
- Color of Grayish white and transparency.
- Their surface structure is coil-like or spring-like, and they look like a mulberry when they are gathered.
- A mulberry body looks similar to a fat globule, but it is easy to distinguish these two by the difference in their surface structures.
- Fully Automated Urine Cell Analyzer can not be detected mulberry bodies.

- Mulberry bodies is not dyed by fat staining.

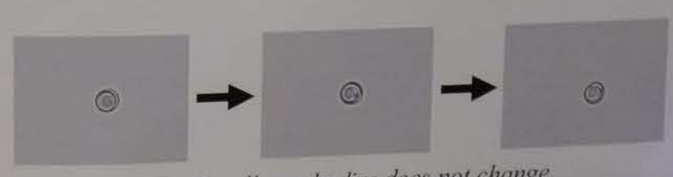


Fig.3 mulberry bodies does not change by hydrochloric acid addition.

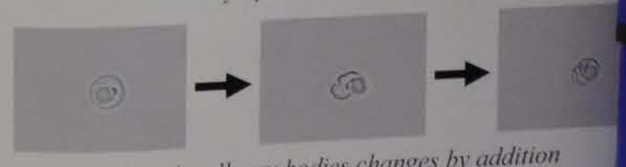


Fig.4 mulberry bodies changes by addition of the sodium hydroxide a little.

Sensitivity and Specificity of Urinary segiments of mulberry bodies

※ Data of Osaka University Hospital

		Mulberry bodies (+)	Mulberry bodies (−)
Fabry ddisease	(+)	125	24
	(−)	1	21

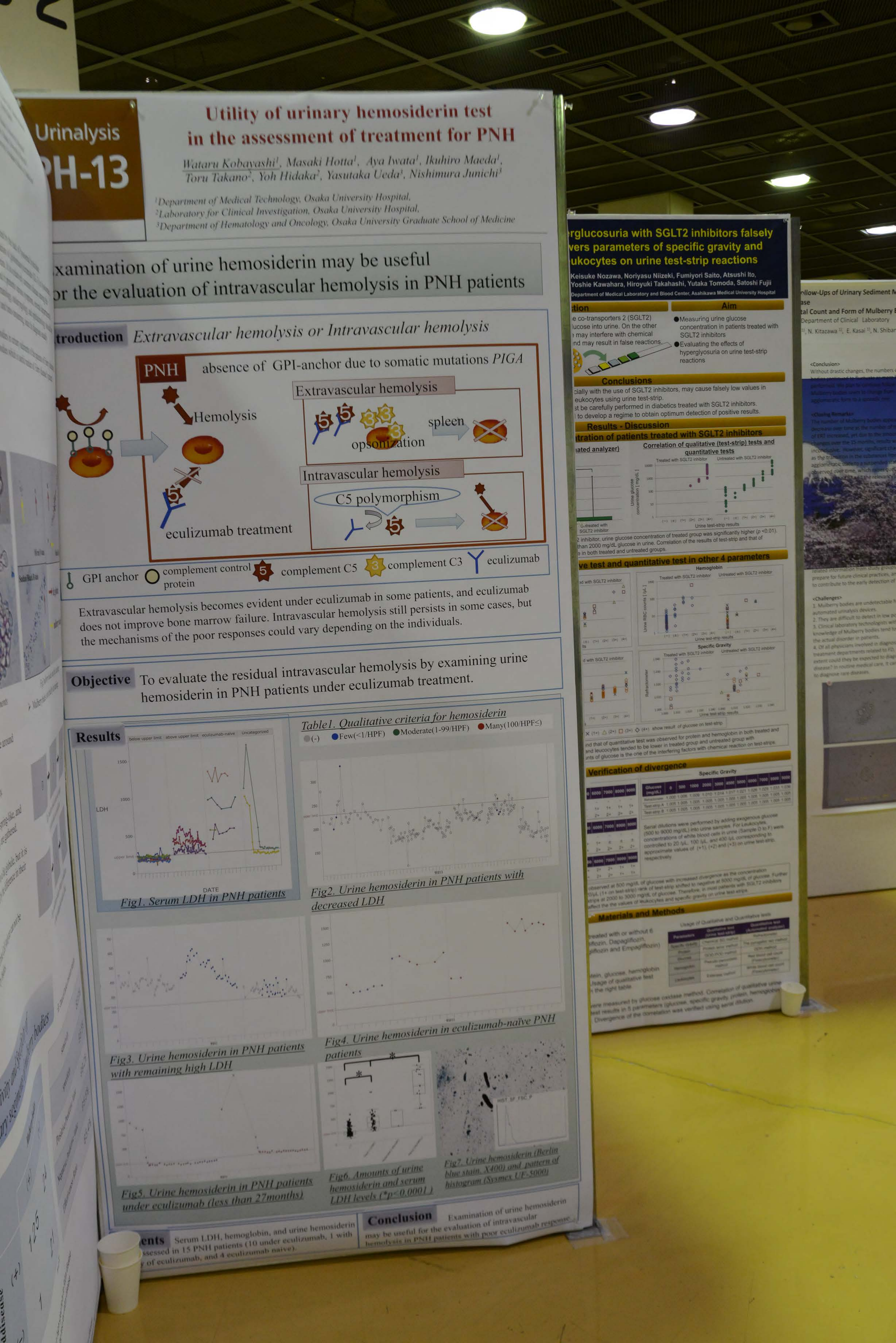
n=171

sensitivity	83.9%
specificity	95.5%
Positive Predictive Value	99.2%
Negative Predictive Value	95.5%
Effectiveness Ratio	85.4%

- Detection of mulberry bodies has a high specificity to Fabry disease
- Urinary sediment is useful as a screening test for Fabry disease

Conclusion

- Mulberry bodies appear in the urine from the most of the patients of Fabry disease.
- Detection of mulberry bodies in the urinary sediments is screening of Fabry disease in high specificity and sensitiv
- Thus, detecting mulberry bodies in the urinary sediments is the simplest way to screen Fabry disease.


Urinalysis
H-13
Utility of urinary hemosiderin test
in the assessment of treatment for PNH
Wataru Kobayashi[1], Masaki Hotta[1], Aya Iwata[1], Ikuhiro Maeda[1],
Toru Takano[2], Yoh Hidaka[2], Yasutaka Ueda[3], Nishimura Junichi[3]
[1]Department of Medical Technology, Osaka University Hospital,
[2]Laboratory for Clinical Investigation, Osaka University Hospital,
[3]Department of Hematology and Oncology, Osaka University Graduate School of Medicine
or the evaluation of intravascular hemolysis in PNH patients
Extravascular hemolysis or Intravascular hemolysis
PNH
absence of GPI-anchor due to somatic mutations PIGA
Hemolysis
eculizumab treatment
Extravascular hemolysis
spleen
opsonization
Intravascular hemolysis
C5 polymorphism
GPI anchor
complement control protein
complement C5
complement C3
eculizumab
Extravascular hemolysis becomes evident under eculizumab in some patients, and eculizumab does not improve bone marrow failure. Intravascular hemolysis still persists in some cases, but the mechanisms of the poor responses could vary depending on the individuals.
Objective
To evaluate the residual intravascular hemolysis by examining urine hemosiderin in PNH patients under eculizumab treatment.
Results
Table1. Qualitative criteria for hemosiderin
(-)
Few(<1/HPF)
Moderate(1-99/HPF)
Many(100/HPF≤)
LDH
DATE
Fig1. Serum LDH in PNH patients
Fig2. Urine hemosiderin in PNH patients with decreased LDH
Fig3. Urine hemosiderin in PNH patients with remaining high LDH
Fig4. Urine hemosiderin in eculizumab-naïve PNH patients
Fig5. Urine hemosiderin in PNH patients under eculizumab (less than 27months)
Fig6. Amounts of urine hemosiderin and serum LDH levels (*p<0.0001)
Fig7. Urine hemosiderin (Berlin blue stain, X400) and pattern of histogram (Sysmex UF-5000)
Serum LDH, hemoglobin, and urine hemosiderin
ssessed in 15 PNH patients (10 under eculizumab, 1 with
of eculizumab, and 4 eculizumab naive).
Conclusion
Examination of urine hemosiderin
may be useful for the evaluation of intravascular
hemolysis in PNH patients with poor eculizumab response
Keisuke Nozawa, Noriyasu Niizeki, Fumiyori Saito, Atsushi Ito,
Yoshie Kawahara, Hiroyuki Takahashi, Yutaka Tomoda, Satoshi Fujii
Department of Medical Laboratory and Blood Center, Asahikawa Medical University Hospital
Aim
Measuring urine glucose concentration in patients treated with SGLT2 inhibitors
Evaluating the effects of hyperglyosuria on urine test-strip reactions
Conclusions
Results - Discussion
Correlation of qualitative (test-strip) tests and quantitative tests
Verification of divergence
Materials and Methods

Urinalysis

PH-14

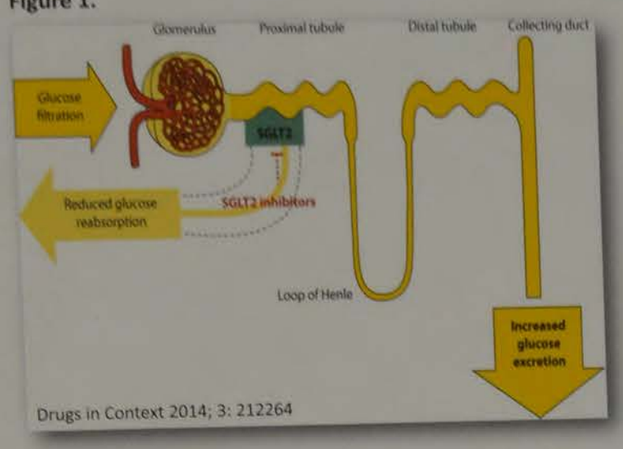
The sodium glucose co-transporter 2 receptor inhibitors, the newest class of drugs for type 2 diabetes, attenuated the results of leukocytes tested by urine dipsticks

Keita Kamiyama, Takao Kimura, Tetsuo Machida, Masami Murakami

Clinical Laboratory Center, Gunma University Hospital, Japan; Department of Clinical Laboratory Medicine, Gunma University Graduate School of Medicine, Maebashi, Gunma, Japan; Gunma University Graduate School of Health Sciences, Maebashi, Gunma, Japan.

Background: The sodium glucose co-transporter 2 receptor (SGLT-2) inhibitors are the newest class of drugs for type 2 diabetes. Inhibition of SGLT-2 results in a lowering of renal glucose reabsorption and an increase in urinary glucose excretion, with a related reduction of plasma glucose levels(Figure1). Women taking the SGLT2 inhibitors have increases in urinary tract and genital tract infection. The influence of SGLT-2 inhibitors on urinalysis is not clear.

Figure 1.



Objective: In this study we investigated the influence of the SGLT-2 inhibitors on urinalysis.

Subjects and Methods: We performed the urinalysis of 60 diabetics treated with or without of the SGLT-2 inhibitors. Thirty diabetics treated with SGLT-2 inhibitors and 30 diabetics treated without of SGLT-2 inhibitors. We compared the result of urinalysis performed by two independent methods; tested by urine dipsticks and quantitative value measured by automated urine analyzer.

Urinalysis :
Specific gravity , Leukocytes : Uropaper αⅢ 'eiken' , US-3100Rplus , EIKEN CHEMICAL CO., LTD.
Urinary protein , Urinary albumin : μ-TP 2 , μ-Alb auto , Wako Pure Chemical Industries, Ltd.
Urinary creatinine : Cygnas auto CRE , Shino-Test Corporation
Urinary glucose : Pure auto S GUR-R,SEKISUI MEDICAL CO., LTD.

Results:

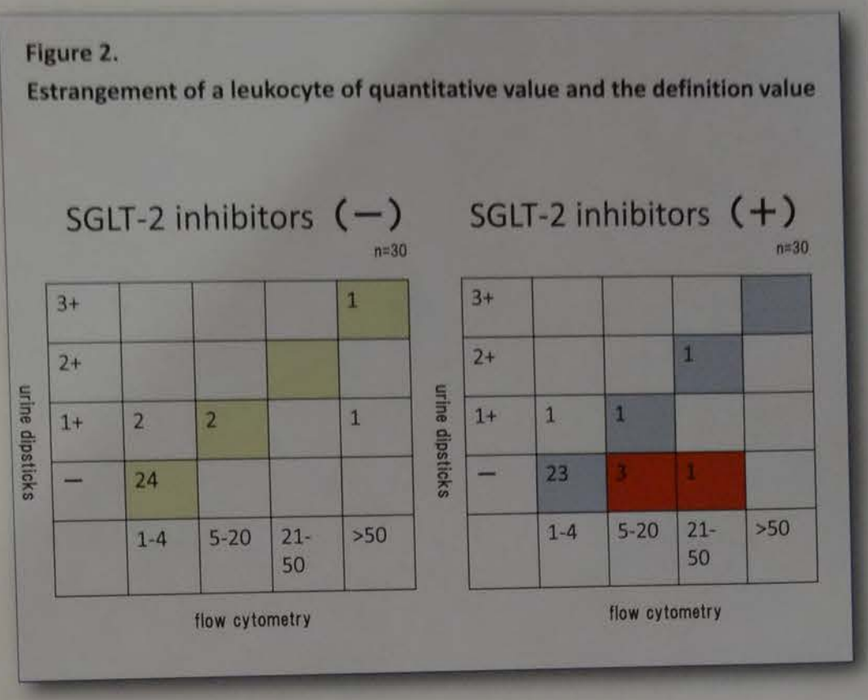
1. The result of urinary protein, protein/creatinine (P/C) ratio, albumin, albumin/creatinine (A/C) ratio and glucose were compatible with the value tested by urine dipsticks and quantitative value measured by automated chemical analysis(Table1).

Table 1.
Rate of concordance between dipsticks and quantitative value in diabetic patients treated with or without of SGLT-2 inhibitors

	SGLT-2 inhibitors (-) (n=30)	SGLT-2 inhibitors (+) (n=30)
Urinary glucose	86.7 %	96.7 %
Urinary protein	90 %	100 %
Urinary albumin	83.3 %	86.7 %
Urinary creatinine	66.7 %	80 %
Red blood cell	80 %	90 %
White blood cell	90 %	83 %
Protein/creatinine ratio	76.7 %	70 %
Albumin/creatinine ratio	70 %	73.3 %

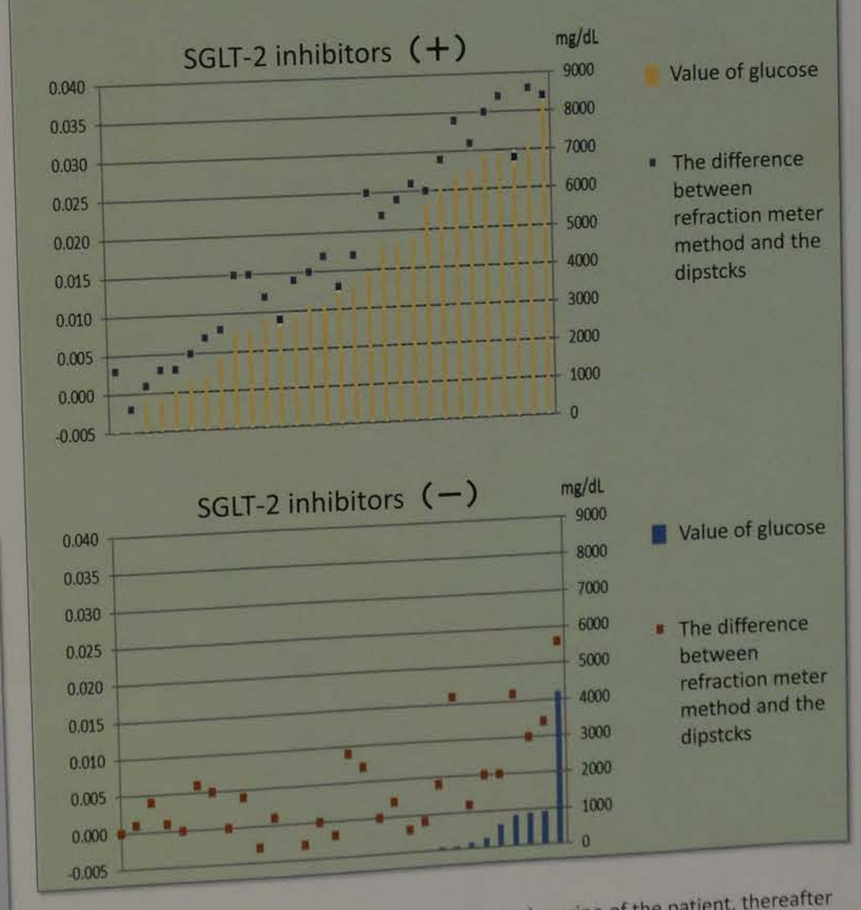
2. The value of leukocytes tested by urine dipsticks was significantly lower than that of quantitative value measured by fluorescent flow cytometry (Figure 2).

Figure 2.
Estrangement of a leukocyte of quantitative value and the definition value



3. The value of specific gravity tested by urine dipsticks was significantly lower than that of quantitative value measured by the refraction method. High dose of urinary glucose did not attenuate the specific gravity tested by urine dipsticks(Figure 3).

Figure 3. Comparison of refraction method and urine dipsticks



4. We added various concentrations of glucose in the urine of the patient, thereafter urine analysis was performed by urine dipsticks and by fluorescent flow cytometry. The result of leukocytes tested by urine dipsticks, but not by fluorescent flow cytometry, was attenuated by glucose in concentration dependent manner.

Conclusion: The value of specific gravity and leukocytes tested by urine dipsticks was attenuated by high concentration of urinary glucose in diabetic patients treated by SGLT2 inhibitors. The high level of urine glucose caused the false-negative test of a leukocytes tested with an urine dipstick and dissociation of specific gravity tested by urine dipsticks and refraction method.

Urinalysis

PH-15

Hyperglucosuria with SGLT2 inhibitors falsely lowers parameters of specific gravity and leukocytes on urine test-strip reactions

Keisuke Nozawa, Noriyasu Niizeki, Fumiyori Saito, Atsushi Ito, Yoshie Kawahara, Hiroyuki Takahashi, Yutaka Tomoda, Satoshi Fujii

Department of Medical Laboratory and Blood Center, Asahikawa Medical University Hospital

Introduction

Diabetics treated with sodium-glucose co-transporters 2 (SGLT2) inhibitors excrete large amounts of glucose into urine. On the other hand, co-existing substances in urine may interfere with chemical reaction on test-strips for urinalysis and may result in false reactions.

Aim

- Measuring urine glucose concentration in patients treated with SGLT2 inhibitors
- Evaluating the effects of hyperglyosuria on urine test-strip reactions

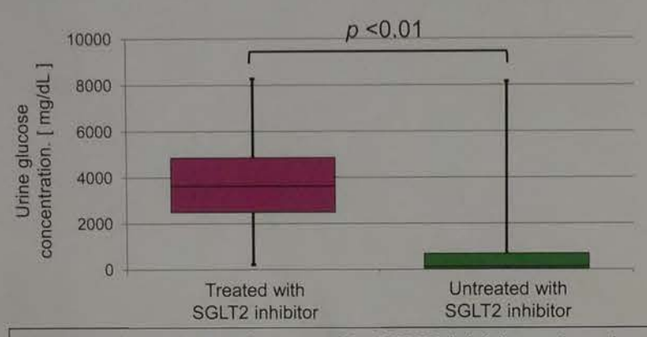
Conclusions

- Hyperglycosuria of diabetics, especially with the use of SGLT2 inhibitors, may cause falsely low values in parameters of specific gravity and leukocytes using urine test-strip.
- Urinalysis with urine test-strips must be carefully performed in diabetics treated with SGLT2 inhibitors.
- Our study suggests imminent need to develop a regime to obtain optimum detection of positive results.

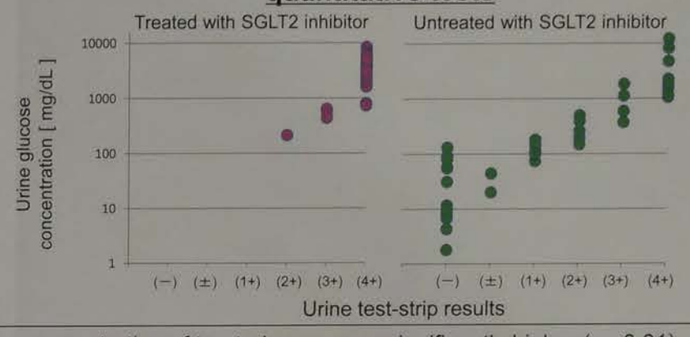
Results - Discussion

Urine glucose concentration of patients treated with SGLT2 inhibitors

Using quantitative tests (automated analyzer)

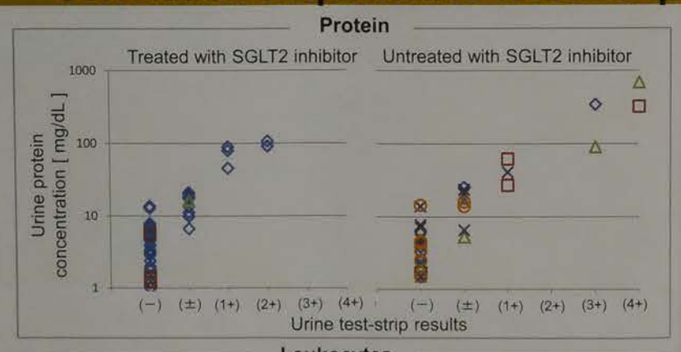


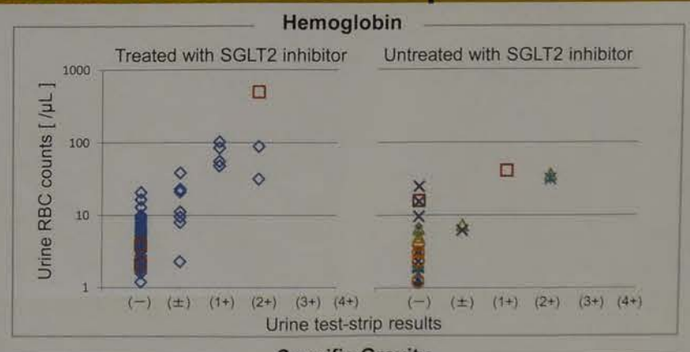
Correlation of qualitative (test-strip) tests and quantitative tests

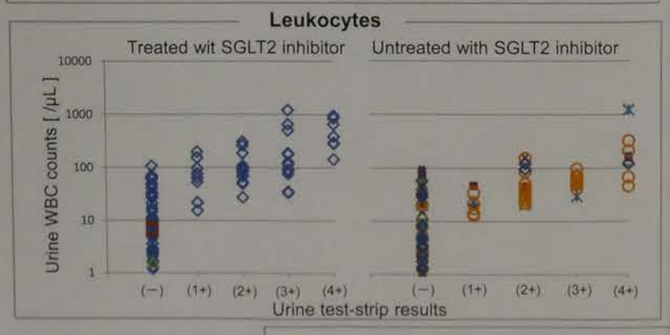


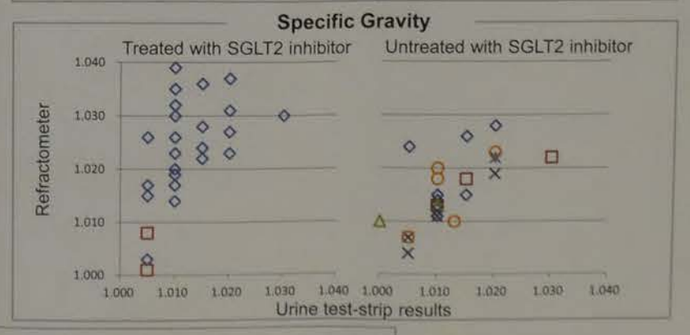
Compared to untreated group with SGLT2 inhibitor, urine glucose concentration of treated group was significantly higher (p <0.01). Over 75% of the patients exhibited more than 2000 mg/dL glucose in urine. Correlation of the results of test-strip and that of quantitative test was observed for glucose in both treated and untreated groups.

Correlation of qualitative test and quantitative test in other 4 parameters









○ (−) ✳ (±) × (1+) △ (2+) □ (3+) ◇ (4+) show result of glucose on test-strip

Close correlation of the results of test-strip and that of quantitative test was observed for protein and hemoglobin in both treated and untreated groups. Values in specific gravity and leucocytes tended to be lower in treated group and untreated group with hyperglycosuria using test-strip. Large amounts of glucose is the one of the interfering factors with chemical reaction on test-strips.

Verification of divergence

Leukocytes

Urine test-strip A

Glucose (mg/dL)	0	500	1000	2000	3000	4000	5000	6000	7000	8000	9000
Sample D	1+	1+	1+	1+	1+	1+	-	-	-	-	-
Sample E	2+	2+	2+	2+	2+	2+	2+	1+	1+	1+	1+
Sample F	3+	3+	3+	3+	2+	2+	2+	2+	2+	2+	2+

Urine test-strip B

Glucose (mg/dL)	0	500	1000	2000	3000	4000	5000	6000	7000	8000	9000
Sample D	1+	1+	1+	1+	±	±	-	-	-	-	-
Sample E	2+	2+	2+	2+	1+	1+	1+	1+	±	±	±
Sample F	3+	3+	3+	3+	2+	2+	2+	2+	2+	2+	2+

urine test-strip C

Glucose (mg/dL)	0	500	1000	2000	3000	4000	5000	6000	7000	8000	9000
Sample E	2+	2+	2+	2+	2+	2+	2+	2+	2+	1+	1+
Sample F	3+	3+	3+	3+	2+	2+	2+	2+	2+	1+	1+

Specific Gravity

Glucose (mg/dL)	0	500	1000	2000	3000	4000	5000	6000	7000	8000	9000
Refractometer	1.000	1.008	1.009	1.010	1.014	1.017	1.021	1.026	1.029	1.033	1.036
Test-strip A	1.005	1.005	1.005	1.005	1.005	1.005	1.005	1.005	1.005	1.005	1.005
Test-strip B	1.005	1.005	1.005	1.005	1.005	1.005	1.005	1.005	1.005	1.005	1.005

Serial dilutions were performed by adding exogenous glucose (500 to 9000 mg/dL) into urine samples. For Leukocytes, concentrations of white blood cells in urine (Sample D to F) were controlled to 20 /μL, 100 /μL, and 400 /μL corresponding to approximate values of (+1), (+2) and (+3) on urine test-strip, respectively.

With specific gravity shift to 1 lower rank was observed at 500 mg/dL of glucose with increased divergence as the concentration increased. With leukocytes concentration of 20/μL (1+ on test-strip) rank of test-strip shifted to negative at 5000 mg/dL of glucose. Further shift to 1 lower rank was found on some test-strips at 2000 to 3000 mg/dL of glucose. Therefore, in most patients with SGLT2 inhibitors the administration of this agent may critically affect the the values of leukocytes and specific gravity on urine test-strips.

Materials and Methods

Samples

229 urine samples from diabetics treated with or without 6 classes of SGLT2 inhibitors (Ipragliflozin, Dapagliflozin, Luseogliflozin, Tofogliflozin, Canagliflozin and Empagliflozin) were obtained.

Parameters and Analysis

5 parameters (specific gravity, protein, glucose, hemoglobin and leukocytes) were evaluated. Usage of qualitative test and quantitative test was shown in the right table.

Usage of Qualitative and Quantitative tests

Parameters	Qualitative test (Urine test-strip)	Quantitative test (Automated analyzer)
Specific Gravity	Chemical SG method	Refractometer
Protein	Protein error method	The pyrogallol red method
Glucose	GOD-POD method	GDH method
Hemoglobin	Pseudo-peroxidase method	Red blood cell count (Flowcytometer)
Leukocytes	Esterase method	White blood cell count (Flowcytometer)

Methods

Concentrations of urine glucose were measured by glucose oxidase method. Correlation of qualitative urine test-strip results and quantitative test results in 5 parameters (glucose, specific gravity, protein, hemoglobin and leukocytes) were determined. Divergence of the correlation was verified using serial dilution.

A-2 A-2

Urinalysis

PH-16

A case of Non-IgE mediated Gastrointestinal Food Allergy in neonate

N.Tsujimoto,A.Shimonosono,M.Tsuzaki,K.Miura.

Department of Clinical Laboratory Amagasaki Co-op Hospital,Japan

Background

Non-IgE mediated gastrointestinal food allergy in neonates and infants is known to occur after being exposed to powder milk or mother's milk in neonate or infant term. Patients frequently shows symptoms of vomiting melena, however non-specific manifestation of poor sucking, torpidity, and poor increase of body mass makes it **difficult to diagnose**[1].

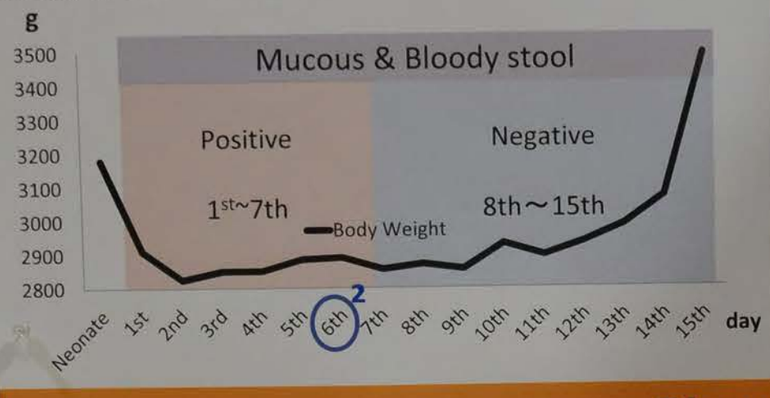
This is a case report of non-IgE gastrointestinal food allergy, which stool smear led an early diagnosis.

Differential diagnosis[1]

Necrotizing enterocolitis	Cytomegalovirus colitis
Gastrointestinal obstruction	Lymphoid hyperplasia
Bacterial enteritis	Meckel's diverticulum
Pseudomembranous enterocolitis	Intetinal malrotation
Hemolytic-uremic syndrome	Bowel intussusception
Parasitic disease	Pyloric stenosis
Lactose intolerance	Hirschsprung('s) disease
Neonatal melena	Early onset Cron's disease
Blood in stool from mother's milk	Early onset ulcerative colitis

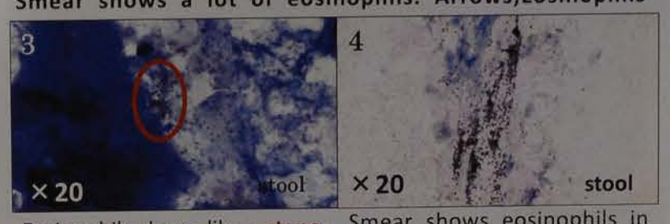
Case

Male neonate, who was born in good condition,at 35 weeks and 3 days of gestational age. Mucous and bloody stool was found on postnatal day 2, which was persistent, but had been disappeared after **switching to allergy milk**[2], after suspecting non-IgE mediated gastrointestinal food allergy corroborated by stool smear, in neonate term.



Cytological findings

Smear shows a lot of eosinophils. Arrows;Eosinophils



Eosinophils shows like a **stone-wall arrangement.**

Smear shows eosinophils in clusters and scattered singly,with many types of bacteria on the background.

May-Grünwald's-Gimsa's staining(1~4)

Discussion

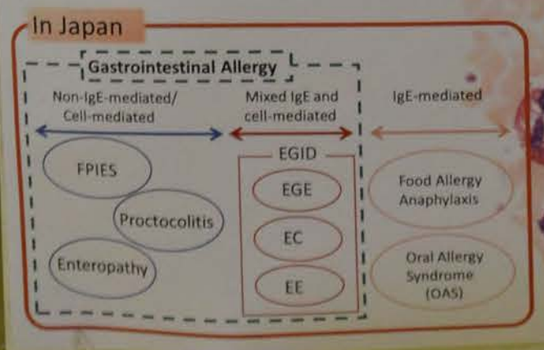
Delayed diagnosis of non-IgE mediated gastrointestinal food allergy in neonates and infants might result in ileus and affect developmental disability. In general definite diagnosis should follow in **clinical entities**[3] to examine (**Allergen-specific lymphocyte stimulation test ; ALST**[4])and milk allergy tolerance test. However stool smear is useful so as not to delay treatment.

Clinical entities[3]

In western countries

Ⅰ.Food Protein-Induced Enterocolitis Syndrome(FPIES)
Ⅱ. Food Protein-Induced Proctocolitis Syndrome(Proctocolitis)
Ⅲ. Food Protein-Induced Enteropathy Syndrome(Enteropathy)
Ⅳ.Celiac Disease
Ⅴ.Eosinophilic Esophagitis(EoE)
Ⅵ.Eosinophilic Gastroenteritis(EGE)

In Japan



Allergen-specific lymphocyte stimulation test; ALST[4]

Test Item	Result				
	Count (cpm)	SI	Decision	Reference interval	Cut of index
κ-Casein	909	2.07	(+)	1.58	1.3
Lactoferrin	1130	2.57	(−)	2.62	1.0
Human-α-lactalbumin	154	0.35	(−)	2.27	0.2

Conclusion

This case was diagnosed type I allergy after IgE and RAST(milk) resulted positive, **6 months later***

Stool smear of initial stage is thought to be useful for prevention of serious condition.

Laboratory findings					
Test Item	Neonate	6months later*	9months later	18months later	Reference intervals
IgE(RIST)	2>U/ml	16U/ml	66U/ml	119U/ml	0~170U/ml
IgE(RAST)					
Milk	0.00	0.36	0.38	0.65	0~0.34UA/ml
Casein	0.00	0.05	0.06	0.14	
α-lactalbumin	0.00	0.21	0.28	0.07	
β-lactglobulin	0.01	0.11	0.08	0.02	
Beef	NT	0.16	0.15	0.65	
Egg albumen	0.00	0.00	0.00	NT	

Urinalysis

PH-17

The Importance of Follow-Ups of Urinary Sediment Mulberry Bodies in Fabry Disease

Transitions in the Total Count and Form of Mulberry Bodies

Nagano Chuo Hospital, Department of Clinical Laboratory

A. Murata [1], S. Kusano [1], N. Kitazawa [1], E. Kasai [1], N. Shibano [1]

<Introduction>

Fabry disease (FD), is a fatal inherited metabolic disease of X-linked recessive mode of inheritance that exhibits various clinical symptoms which result from the accumulation of glycolipid globotriaosylceramide (GL-e) in cells of the entire body (mostly microvascular endothelia cells), the accumulation of which results from reduced activity or amount of the hydrolytic enzyme a-galactosidase (α-Gal) present in lysosome.

During childhood, symptoms include pain in extremities, burning sensation, decreased sweating, and angiokeratoma. In adulthood, the disease presents vascular lesions in the kidneys, brain, and heart, and in early cases can progress to renal failure in patients in their 20s, which may lead to death in their 40s to 50s. Although it is an X-linked recessive hereditary disorder, even female heterozygous carriers can share the same severe symptoms as classical FD males.

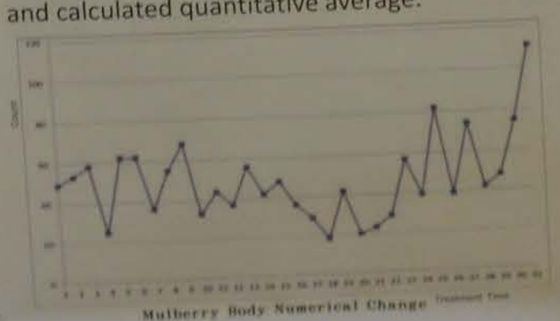
Paying close attention to the Mulberry bodies (cells and bodies) found in the urinary sediments from a Fabry disease patient from our hospital, we reported the transitions in the total count and form, etc., of the Mulberry bodies during the 15 month enzyme replacement therapy (ERT) (agalsidase beta 1 mg/kg).

< Patient Background (At the time of diagnosis: September 2014)>

26-year-old male. Height: 165 cm.
Weight: 52.5 kg
Onset: Pain in extremities, approximately from the age of 5.
Physical finding: Angiokeratoma.
Laboratory findings : Left ventricular hypertrophy (Electrocardiogram, Chest X-ray, Echocardiography)
Family history : Two cousins on the mother's side have FD. (Ongoing ERT)
Enzyme activity: Dry Blood Spot α-GAL activity 3.3 Agal U (Cutoff: >15.0 Agal U)
Accumulated material: Plasma GL-3 value 12.8 μg /ml (Normal value <7 μg/ml)

<Method>

Observation period : December 2014 to March 2016 (Every two weeks)
Counting method : Sampled from urine before the ERT. Urine sediment(non-stained), High-power (400 times), 10 fields of view, repeated 3 times and calculated quantitative average.



<Conclusion>

Without drastic changes, the numbers of Mulberry bodies continued to fluctuate as more ERT were performed. We plan to continue follow-ups as the Mulberry bodies seem to change from an agglomeratic form to a sporadic one.

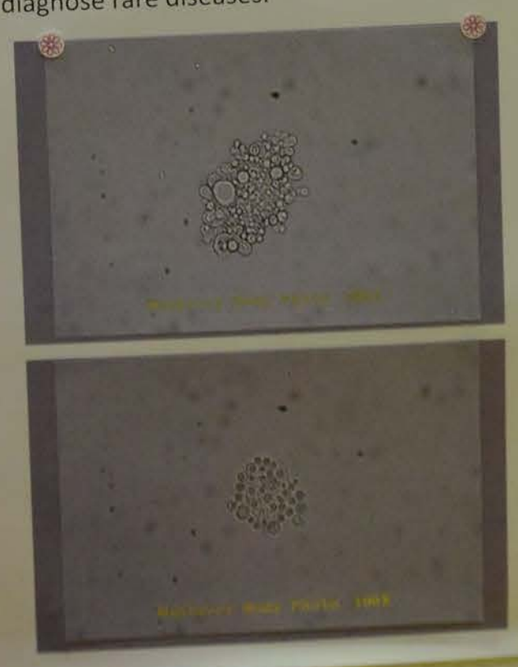
<Closing Remarks>

The number of Mulberry bodies appeared to decrease over time as the number of treatments of ERT increased, yet due to the amount of changes over the 15 months, results were inconclusive. However, significant changes such as the transition in the substances from an agglomeratic state to a suspended state were observed over time, which raises questions on the efficacy of the ERT and the relevancy it may hold. We will continue with the follow-ups on the number and form of the Mulberry bodies, along with any clinical manifestations. The method of counting Mulberry bodies is yet to be established, and so we intend to utilize a Neubauer hemocytometer when a new FD patient is discovered.

Some hereditary diseases are prone to be regional, and in Japan's case, it seems that many FD patients reside in the Nagano and Niigata area. From such environments, we would like to obtain related information from study groups, in order to prepare for future clinical practices, and we hope to contribute to the early detection of FD.

<Challenges>

1. Mulberry bodies are undetectable from automated urinalysis devices.
2. They are difficult to detect in low power fields.
3. Clinical laboratory technologists with no knowledge of Mulberry bodies tend to overlook the actual disorder in patients.
4. Of all physicians involved in diagnosis and treatment departments related to FD, to what extent could they be expected to diagnose this disease? In routine medical care, it can be difficult to diagnose rare diseases.



<Contact information>

Nagano chuo Hospital

E-mail : hosp@healthcoop-nagano.or.jp