Editorial: Letter from the Editor-in Chief

〔Lab Med Int 2022; 1(1): 1-2〕

Laboratory Medicine International is inaugurated with great expectations that many will enjoy the new international journal of Laboratory Medicine.

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On behalf of the Editors, Editorial Board members, and journal staff, I wish to warmly welcome you to the inaugural edition of Laboratory Medicine International (LMI). LMI is a peer-reviewed international journal published quarterly by the Japanese Society of Laboratory Medicine. The aim of this editorial is to welcome readers and provide a short overview of the concept and content of this peer-reviewed publication.

The primary scope of LMI covers clinical chemistry, immunology, microbiology, hematology, science of thrombosis and hemostasis, transfusion medicine, molecular diagnostics, cytology, histology, therapeutic drug monitoring and toxicology, laboratory management and informatics, and laboratory administration and management. In principle, original research papers, reviews, case reports, and editorials are considered for publication. LMI also considers publications for clinically relevant laboratory topics on laboratory methods, evidence-based laboratory medicine, health economics, best practices, test utilization, critical care based on the scientific scope of laboratory medicine, and thoughtful commentary on the practice of laboratory medicine. Case Reports will be a regular LMI feature; for many cases, there will be findings of interest to members of the laboratory community, possibly leading to further developments of laboratory medicine. Paper submission to LMI is conducted using the ScholarOne Manuscripts system.

The information in LMI will consist of findings and applications available in clinical practice and laboratory medicine both at present and in the future. Publications of contributions submitted to LMI will be timely and efficiently handled and accessible to the scientific community expeditiously. Contributions will be available online only after they have been approved by both the LMI editorial staff and authors.

Again, we are excited and delighted to introduce LMI, a new high-quality, peer-reviewed, international open-access journal. Papers, including original research, new methods and critical evaluations, case reports, and short reports in the fields of laboratory medicine, published in LMI are presented by J-STAGE, a platform for scholarly publications, operated by the Japan Science and Technology Agency (JST). We also welcome papers which describe critical evaluations of biomarkers and their role in the diagnosis and treatment of clinically significant disease, validation of commercial and in-house methods of in vitro diagnostics, method comparison studies, interference reports, the development of new reagents and reference materials, reference range studies, and regulatory compliance reports. Manuscripts describing the development of new methods applicable to laboratory medicine, including point-of-care testing, are also encouraged, even if the submitted paper is a report from preliminary or small-scale studies. Many laboratory-developed tests were created in response to unmet needs, e.g., for rare diseases where limited laboratory medicine resources are available. Notwithstanding, a critical point is proper validation that these assays are safe and effective for clinical use.

We hope that LMI will meet the needs of the laboratory medicine community by providing timely, practical infor-

mation on the challenges which face all of us daily in delivering optimal patient care through laboratory testing. The forward-looking path for laboratory medicine will involve strategies for collaborating, communicating, and integrating with all healthcare stakeholders. There are many ongoing efforts and activities worldwide. All those involved with LMI are looking forward to engaging users and contributors to this LMI resource and playing an active role in the evolution of laboratory medicine.

Editorial: Message from the President

〔Lab Med Int 2022; 1(1): 3〕

Celebrating LMI Inaugural Issue

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With this commemorative inaugural issue, the Japanese Society of Laboratory Medicine (JSLM) is proud to launch Laboratory Medicine International (LMI), a new journal dedicated to the advancement of the science and practice of laboratory medicine and clinical pathology.

Medical and laboratory tests have long been the cornerstone of diagnosis and decision-making in clinical practice, and the dependence on laboratory tests has continued to grow in the modern era of medicine. The development of novel molecular technology has further enabled the application of genetic analysis to diagnosis, embodying the understanding of human diseases through the most fundamental etiology. Laboratory medicine covers all areas of diagnosis and promotes patient care in scientific ways, organizing knowledge in the form of testable explanations and predictions about human beings. However, the clinical application of laboratory tests to patient diagnosis and the subsequent selectoin of therapeutic strategies has yet to be fully investigated, from conventional fields like specimen sampling to the latest evolved fields like molecular diagnosis. Under these circumstances, LMI will be a premium resource for those interested in improving patient care through accurate and reliable medical tests.

We are thrilled to have Hiroshi Yoshida, MD, PhD, as founding Editor-in-Chief of LMI. Dr. Yoshida is a professor of laboratory medicine, and internal medicine specializing in lipid metabolism and atherosclerosis, and Director of the Jikei University Kashiwa Hospital. He will surely bring wisdom gained from his over 30 years of pioneering research in laboratory medicine and over 10-year experience in editing the Japanese Guidelines of Laboratory Medicine, to present -day challenges: how to implement useful medical tests in real-world healthcare from a scientific standpoint. He is definitely the right person for the job.

LMI also enjoys the support of the outstanding group of Deputy Editor Dr. Tatsuo Shimosawa, associate editors, and editorial board members who bring multidisciplinary expertise and a wealth of experience to the journal, which is essential in the field of laboratory medicine that covers all areas of modern medicine. We applaud the efforts of the founding editors for the launch of LMI, a longtime dream that our society has pursued.

The importance of laboratory medicine has never been recognized so profoundly and realistically as in the current situation caused by the COVID-19 pandemic. Admirably, a respectable predecessor of JSLM once stated: "all medical researches lead to laboratory medicine". We urge our members and others interested in medical and laboratory tests to join LMI and contribute their original work, thoughtful review papers, and efforts as peer reviewers. Such contributions will help overcome threats to worldwide public health through unrestrained scientific enthusiasm to understand the nature of human diseases.

We appreciate all readers for joining us in the launch of LMI.

Editorial

〔Lab Med Int 2022; 1(1): 4-5〕

Is pre-heparin hepatic triglyceride lipase useful to understand the mechanisms of dyslipidemia?

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In the paper entitled "Association of pre-heparin plasma hepatic triglyceride lipase with lipoprotein subspecies in type 2 diabetes, Comparison with lipoprotein lipase" in this issue of the Laboratory Medicine International, Nagaike et al. measured pre-heparin hepatic triglyceride lipase (HTGL) as well as pre-heparin lipoprotein lipase (LPL) in 120 Japanese diabetic subjects [1].

The absolute levels of HTGL and LPL are usually evaluated after injection with heparin, because HTGL and LPL exist on the surface of the liver sinusoidal capillaries [2] and capillary endothelial cells [3], respectively. To investigate the existence of gene deletion or heterogenous insufficiency in the patients with severe hypertriglyceridemia, the evaluation of total mass of HTGL and LPL is important. However, HTGL and LPL should be involved in the pathogenesis of dyslipidemia in patients with milder hypertriglyceridemia. Moreover, the activities of lipases have been reported to be lower in diabetic patients [4] who usually suffer from dyslipidemia characterized with high remnant lipoproteins and low HDL [5]. Therefore, the investigation on the association of these lipases and lipoprotein profiles has been expected to understand the mechanisms of dyslipidemia and to consider future application of laboratory tests of the lipases for the diagnosis of dyslipidemia.

In this study, the authors investigated pre-heparin HTGL and LPL level instead of post-heparin HTGL and LPL activities. Although this point may be a major limitation in the interpretation of the results to understand the mechanisms for dyslipidemia observed in diabetic patients, I think that this question is worth investigating since the usefulness of pre-heparin HTGL and LPL levels in clinical laboratory medicine is a controversial at present. They demonstrated that pre-heparin HTGL levels had no significant association with remnant-like particle (RLP)-C or subspecies of LDL-C and HDL-C, while pre-heparin LPL levels had significant a positive association with RLP-C and negative ones with HDL-C. They hypothesized that the weaker associations of HTGL with lipoprotein fractions might be due to the fact that in pre-heparin conditions, smaller percentage of HTGL mass was detected compared with LPL [6] [7].

Although this study could not demonstrate the usefulness of pre-heparin HTGL measurement, negative results in this study will be important when specific modulation of pre-heparin HTGL levels in some unique pathological conditions will be identified in the future. I expect future studies with primary dyslipidemia caused by genetic factors such as familial combined hyperlipidemia and moderate hypertriglyceridemia caused by alcohol overconsumption.

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Original

Association of pre-heparin plasma hepatic triglyceride lipase with lipoprotein subspecies in type 2 diabetes, Comparison with lipoprotein lipase

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ABSTRACT

Aims: Hepatic triglyceride lipase (HTGL) is an essential enzyme for all lipoprotein metabolism. Recently, a sensitive assay kit for measuring HTGL protein in plasma without using heparin injection (pre-heparin) have become available. We investigated the association between pre-heparin HTGL and lipoprotein subspecies and compared it with lipoprotein lipase (LPL) to explore the clinical relevance of measurements in type 2 diabetes.
Methods: Fasting plasma were obtained from 120 patients with type 2 diabetes. HTGL and LPL concentrations were measured by their respective sandwich ELISA kits. Small dense low-density lipoprotein LDL-cholesterol (sdLDL-C), LDL-triglyceride (TG), and high-density lipoprotein (HDL)3-C was measured using the homogenous methods established by us. HDL2-C was calculated by subtracting HDL3-C from HDL-C. Visceral fat area (VFA) was measured by CT scan.
Results: Concentrations of HTGL and LPL were identical; median (interquartile range) were 57(44-74) and 57 (44-70) ng/mL, respectively, and showed a skewed distribution. Gender, age, VFA, and glucose control were not associated with HTGL levels. The higher quartile of HTGL correlated with the higher LDL-C, lower LDL-TG/LDL-C and lower LDL-TG/apolipoprotein B implying TG-depletion of LDL. There were no significant associations of HTGL with remnant-like particle(RLP)-C or subspecies of LDL-C and HDL-C. Pre-heparin LPL inversely correlated with VFA, TG, RLP-C, LDL-TG, and sdLDL-C/LDL-C, and positively correlated with HDL-C and HDL2-C.
Conclusions: Pre-heparin HTGL was only associated with TG-depletion of LDL but not with major lipoprotein subspecies. Pre-heparin HTGL measurements may have limited clinical relevance compared to LPL in diabetic populations.
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Key Words
pre-heparin plasma, hepatic triglyceride lipase, lipoprotein lipase, type 2 diabetes, low-density lipoprotein subspecies

I. Introduction

HTGL is a serine hydrolase with highest specificity for lipoprotein triglyceride (TG) and phospholipid [1]. HTGL is synthesized in the liver and released into plasma by heparin injection, similar to lipoprotein lipase (LPL)[1]. HTGL activity can be measured in post-heparin plasma (PHP) when LPL is inactivated with protamine, NaCl or an LPL-specific antibody [1)2)]. Although many researchers have long recognized the importance of HTGL, the need

for heparin injection has prevented the measurement of HTGL from being used in general clinical practice. For this reason, attempts have been made to measure lipolytic activities of HTGL and LPL in plasma without using heparin injection (pre-heparin) [3)–5)]. However, these enzymes exist in pre-heparin plasma as inactive form, thus it was difficult to measure these activities precisely. Recently the ELISA system for measurement of HTGL protein was frequently used instead of measurement of its activity [6)–8)]. It has been reported that pre-heparin HTGL highly correlated with PHP-HTGL [8)].

HTGL plays an important role in the remodeling of LDL and HDL particles [1)]. HTGL is inversely related to the buoyancy and size of both LDL and HDL particles. Increased HTGL is associated with smaller and denser LDL (sdLDL) and HDL (HDL3) particles, while decreased HTGL is associated with larger and more buoyant LDL (lbLDL) and HDL (HDL2) particles [9)]. So far it remains to be determined the association of pre-heparin HTGL with LDL and HDL subspecies which has been already examined in PHP-HTGL [10)]. Patients with type 2 diabetes often have high levels remnant lipoproteins, sdLDL and low-HDL2-C [11)], which are all be regulated by the action of HTGL. Therefore, diabetes is a good model for studying pre-heparin HTGL and its association with dyslipidemia.

Lipoprotein lipase (LPL) plays a central role in TG-rich lipoprotein metabolism by the lipolysis of TG and the uptake of these particles into the liver [12)]. The catalytically inactive form of LPL can be detected at fairly high levels in pre-heparin plasma [13)]. The LPL protein concentration was measurable using a sandwich enzyme immunoassay system [14)]. Although the clinical importance of measuring pre-heparin LPL protein is well documented [15)16)], little is known about pre-heparin HTGL. We investigated the association between pre-heparin HTGL and lipoprotein subspecies and compared it with lipoprotein lipase (LPL) to explore clinical relevance of measurement of pre-heparin HTGL in type 2 diabetes.

II. Methods

A. Subjects

The subjects are 120 patients (99 male and 21 female) with type 2 diabetes who were outpatients at Showa University Hospital. Characteristics of diabetic subjects were listed in Table 1. Age was 61 ± 10, and body mass index was 26.7 ± 4.3 (mean ± SD). Twenty-eight (24%) patients were insulin users , and seven patients were treated with glucagon-like peptide-1 receptor agonist (GLP-1RA). Most patients (n = 105) were treated with the following oral anti-diabetes drugs (OADs) alone or in combination: a sulfonylurea, metformin, pioglitazone, dipeptidylpeptidase-4 inhibitor, sodium-glucose cotransporter-2 inhibitor, or α-glucosidase inhibitor. Subjects with hyperlipidemia were treated with statins (n= 53), fibrates (n = 13), ezetimibe (n = 6), or omega-3 fatty acids (n = 14) alone or in combination. The majority of hypertensive patients used anti-hypertensive drugs; angiotensin-I converting enzyme (ACEi) or angiotensin II receptor blockers (ARB) (n = 75), calcium channel blockers (CCB) (n = 53), and diuretics (n = 3), alone or in combination. Ten subjects had history of coronary artery disease, 29 subjects had diabetic retinopathy, and 17 subjects had diabetic nephropathy. In preliminary, HTGL concentrations were measured in 17 (9 male and 8 female) healthy volunteers at age of 37 ± 9 for the comparison with diabetics. They never have been diagnosed with diabetes or any other chronic illness.

Pre-heparin plasma samples were taken in the morning after overnight fasting. HTGL protein was measured by the sandwich ELISA (Human HTGL Assay Kit-IBL, Takasaki, Gumma, Japan) [8)]. The assay coefficients of variation were 4.2–7.1% for pre-heparin plasma, and 2.2–4.9% for PHP [8)]. LPL protein was measured by the sandwich ELISA [17)]. LDL-TG, sdLDL-C, and HD3-C concentrations were measured directly in plasma by the homogeneous methods established by our group [18)–20)]. LbLDL-C was calculated by subtracting sdLDL-C from LDL-C, nonHDL-C was calculated by subtracting HDL-C from total-C (TC). HDL2-C was calculated by subtracting HDL3-C from HDL-C. Estimated glomerular filtration rate (eGFR) was calculated from calibrated serum creatinine using the Chronic Kidney Disease Epidemiology Collaboration equation. The albuminuric stage was assessed by the urinary albumin-to-creatinine ratio (UACR) in spot-urine samples. Apolipoproteins were measured by immuno-terbidimetric assay. Apolipoprotein data was missing in 2 patients.

Visceral fat area (VFA) and subcutaneous fat area (SFA) was measured by CT scan. CT data was available in 98 patients. The study was detailed to all subjects including the volunteers who consented to participate, and a written informed consent form was obtained from all participants prior to the study. This study was approved by the Ethics Committee of Showa University Hospital.

B. Statistics

All continuous variables were expressed as mean ± standard deviation (SD). Differences between groups were examined with either Student's t-test or ANOVA.

The p trend was estimated by Jonckheere-Terpstra trend test. Correlations between variables were evaluated with Pearson's simple linear regression analysis. HTGL, LPL, TG, and UACR showed a skewed distribution. Therefore, log-transformed values were adopted for the linear regression analysis. P-value less than 0.05 was considered statistically significant. Analyses were performed using JMP software version 15 (SAS Institute, Cary, NC, USA).

III. Results

Figure 1 depicts the distribution of HTGL and LPL in 120 patients with type 2 diabetes. Concentrations of HTGL and LPL were identical, and median values (interquartile range) were 57(44-74) and 57 (44-70) ng/mL, respectively. These showed a skewed distribution. Therefore, the log-transformed HTGL or LPL were adopted for subsequent linear regression analysis. In a preliminary study, the HTGL concentration of 17 healthy volunteers was 44.2 ± 16.7 ng / mL, significantly lower than that of diabetics ($p < 0.05$).

Table 1 shows the characteristics of the subjects and the measurements stratified by the HTGL quartile (Q). Overall, the average BMI was greater 25.0 and the visceral fat area (VFA) was over 100 cm^2, indicating visceral fat obesity. Patients with liver and kidney dysfunction were rare. Their blood pressure was well controlled, but their glycemic control was not fully controlled. TC, LDL-C, nonHDL-C, TG, and HDL-C levels in the majority of subjects were within normal limits, probably because most of them were treated with statins or other lipid-lowering agents. There were no significant differences in age, gender, blood pressure, body mass index, VFA, SFA, or glycemic control between quartiles. There were no significant differences in the use of antihypertensive, hypoglycemic, or lipid-lowering drugs between quartiles. AST and ALT tended to decrease with higher HTGL quartiles, and γ-GTP decreased with higher HTGL quartiles. LPL concentrations were similar between quartiles. TC, LDL-C, nonHDL-C, TG, and HDL-C levels were similar between quartiles. SdLDL-C, lbLDL-C, LDL-TG, HDL2-C, HDL3-C, apo AI, AII, B, CII, CIII, and E, and RLP-C are comparable between quartiles. The higher quartiles of HTGL significantly correlated with lower LDL-TG / LDL-C, or lower LDL-TG / apoB.

Figure 2 shows a simple regression analysis between log-HTGL or log-LPL and VFA or SVF (n = 98). Log-HTGL was not correlated with VFA or SFA, while Log-LPL was inversely correlated with VFA, but not with SFA.

Figure 3 shows a simple regression analysis between log-HTGL and LDL subspecies. Log-HTGL was positively correlated with LDL-C, but not with sdLDL-C or lbLDL-C. Log-HTGL did not correlate with LDL-TG, but inversely with LDL-TG / LDL-C or LDL-TG / apo B. HTGL did not correlate with TG, RLP-C, nonHDL-C, HDL-C, or HDL2,3-C (data not shown).

Figure 4 shows a simple regression analysis between log-LPL and various lipid parameters. Log-LPL was inversely correlated with log-TG, RLP-C, and LDL-TG, but not with LDL-C. Log-LPL was not correlated with sdLDL-C, but was inversely correlated with sdLDL-C / LDL-C. Log-LPL was correlated with HDL-C, HDL2-C, and apoAI, but not with HDL3-C. Log-LPL did not correlate with age, BMI, glycemic control, blood pressure, eGFR, log-UACR, nonHDL-C, LDL-TG / LDL-C, LDL-TG / apo B, apo AII, apoCIII, and apoE (data not shown).

IV. Discussion

We investigated the association between pre-hepa-

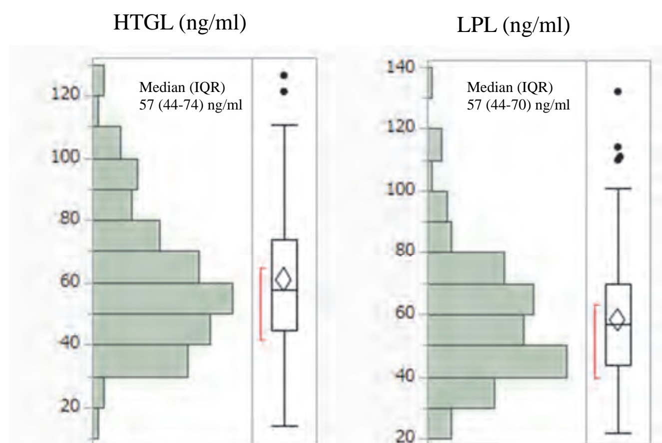

Figure 1 Distribution of hepatic triglyceride lipase (HGL) and lipoprotein lipase (LPL) in pre-heparin plasmas of 120 type2 diabetic patients.

Table 1 Various measurements stratified by quartile of hepatic triglyceride lipase (HTGL) concentration in patients with type 2 diabetes.

	Total	Q1 (14.3-44.8)	Q2 (44.8-57.6)	Q3 (57.6-73.9)	Q4 (74.0-126.5)	p-trend
N (Male/Female)	120 (99/21)	29 (26/3)	31 (24/7)	30 (25/5)	30 (23/7)	ns
HTGL (ng/mL)	61.0 ± 21.9	36.5±6.4	51.3±3.8	63.6±4.8	91.9±13.8	<0.0001
logHTGL (ng/mL)	1.76 ± 0.16	1.55±0.10	1.71±0.03	1.80±0.03	1.96±0.06	<0.0001
Age (years)	61 ± 10	60±11	59±9	64±9	59±9	ns
Anti-diabetic drug(OADs/insulin/GLP-1RA/none)	105/28/7/12 (88%/24%/6%/10%)	24/9/1/4 (83%/31%/3%/14%)	27/6/2/2 (87%/19%/6%/6%)	28/6/3/2 (93%/20%/10%/6%)	26/6/1/4 (87%/20%/3%/13%)	ns
Lipid-lowering drugs (statins/fibrates/ezetimibe/omega3/none)	53/13/6/14/47 (45%/11%/5%/12%/39%)	11/4/2/3 (38%/14%/7%/10%)	15/5/3/7 (48%/16%/10%/23%)	14/1/1/4 (46%/3%/3%/13%)	14/4/2/1 (46%/13%/7%/3%)	ns
Anti-hypertensive drugs (ACEi or ARB/CCB/Diuretics/none)	75/53/3/40 (63%/45%/3%/34%)	18/13/0/11 (62%/45%/0%/38%)	20/12/0/9 (65%/39%/0%/29%)	19/16/2/9 (63%/53%/7%/30%)	19/12/1/11 (63%/40%/3%/37%)	ns
SFA (cm2)#	195 ±97	163±101	193±91	195±91	219±104	ns
eGFR (mL/min/1.73 m2)	80.1 ± 20.6	80 ± 22.3	79.7 ± 21.7	74.2± 16.2	85.9± 20.9	ns
log UACR (mg/g·Cr)	1.4 ± 0.70	1.32±0.65	1.56±0.73	1.38±0.64	1.51±0.76	ns
VFA (cm2)#	171± 64	138±73	173±61	181±58	182±66	ns
SFA (cm2)#	195 ±97	163±101	193±91	195±91	219±104	ns
HbA1c (%)	7.4 ± 1.3	7.95±1.57	7.25±1.32	7.12±0.93	7.34±1.21	ns
LPL(ng/mL)	58.2 ±20.2	59.3±20.1	56.3±21.3	57±21.5	58.0±18.0	ns
Total-C (mg/dL)	185.3 ± 44.2	178.2±40.7	186.5±41.0	187.6±55.7	187.1±39.2	ns
TG (mg/dL)	141.1 ± 96.3	135.3±100.6	157.7±100.3	125.5±75.4	144.9±107.7	ns
logTG(mg/dL)	2.07 ± 0.26	2.03±0.3	2.13±0.25	2.04±0.21	2.07±0.27	ns
LDL-C (mg/dL)	108.8 ± 37.5	98.6±32.0	103.7±32.7	117.5±49.1	113.8±31.6	ns
sdLDL (mg/dL)	36.7 ± 20.8	33.8±19.4	35.6±21.8	36.0±20.5	40.7±21.5	ns
lbLDL (mg/dL)	72.1 ± 31.1	64.7±27.0	68.0±25.5	81.4±37.7	73.0±31.4	ns
LDL-TG (mg/dL)	25.1 ± 6.8	26.5±7.3	25.2±5.7	24.5±9.1	23.6±4.1	ns
HDL-C (mg/dL)	51.2 ± 13.2	53.0±13.5	52.9±15.1	47.5±11.8	51.5±11.6	ns
non HDL-C (mg/dL)	134.3 ± 43.7	124.8±38.4	133.5±39.6	141.4±57.9	135.6±35.7	ns
HDL2-C (mg/dL)	29.8 ± 11.1	31.5±11.1	31.8±13.7	27.7±9.7	28.4±9.1	ns
HDL3-C (mg/dL)	21.4 ± 5.4	21.5±4.7	21.1±6.0	19.8±5.1	23.1±5.1	ns
apo A1 (mg/dL) *	131.1 ± 23.5	136.9± 23.8	134.7± 23.6	120 ± 22.2	133.8 ± 22.0	ns
apo AII (mg/dL) *	29.5 ± 5.1	29.6 ± 4.9	30.3 ± 5.1	26.9 ±3.8	30.2 ± 6.0	ns
apoB (mg/dL) *	91.6 ± 26.7	85.0±22.8	91.5±25.1	96.8±36.0	92.2±20.0	ns
apoCII (mg/dL) *	4.6 ± 1.9	4.59±2.0	4.9±2.1	4.1±1.6	4.7±1.8	ns
apoCIII (mg/dL) *	10.7 ± 5.0	10.2±4.3	12.4±7.0	9.3±3.2	10.5±4.1	ns
apoE (mg/dL) *	4.1 ± 1.2	4.3±1.29	4.45±1.49	3.9±1.09	3.93±0.89	ns
RLP-C (mg/dL)	8.2 ± 7.9	8.1±8.9	9.3±8.9	7.4±6.8	7.8±6.8	ns
LDL-TG/LDL-C	0.25 ± 0.09	0.28±0.08	0.27±0.1	0.23±0.09	0.22±0.06	0.0172
LDL-TG/apoB *	0.28 ± 0.07	0.32±0.07	0.29±0.07	0.27±0.08	0.26±0.06	0.0187

#n = 98, *n = 118. Values are mean ± SD. The p trend was estimated by Jonckheere-Terpstra trend test .
ns = not significance

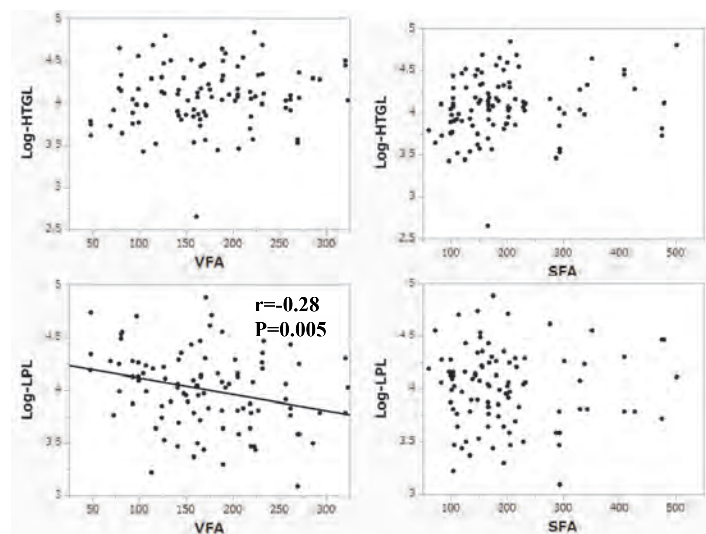

Figure 2 Relationship between log-HTGL or log-LPL and visceral fat area (VFA) or subcutaneous fat are (SFA) in 98 type 2 diabetic patients.

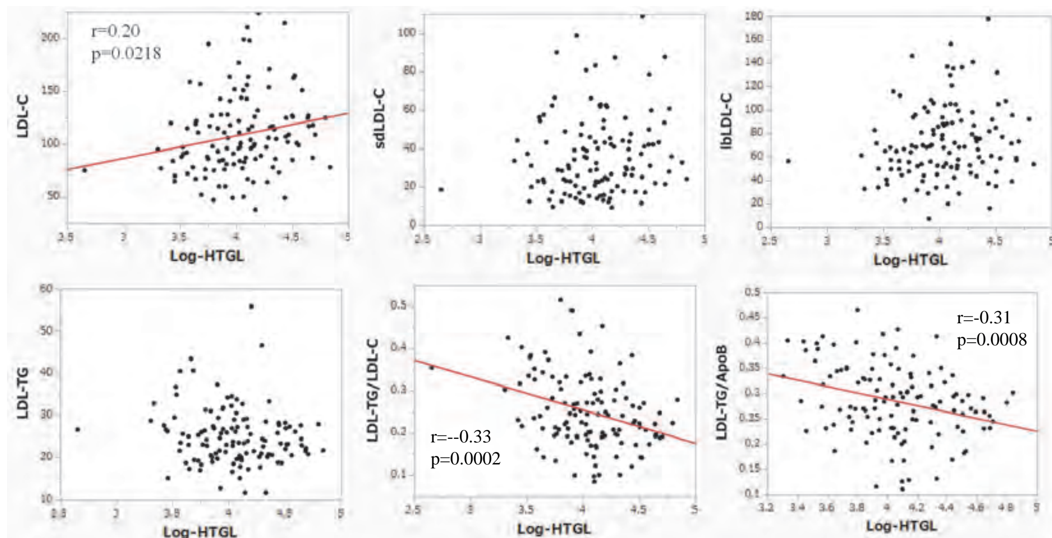

Figure 3 Relationship between log-HTGL and LDL subspecies in type diabetic patients.

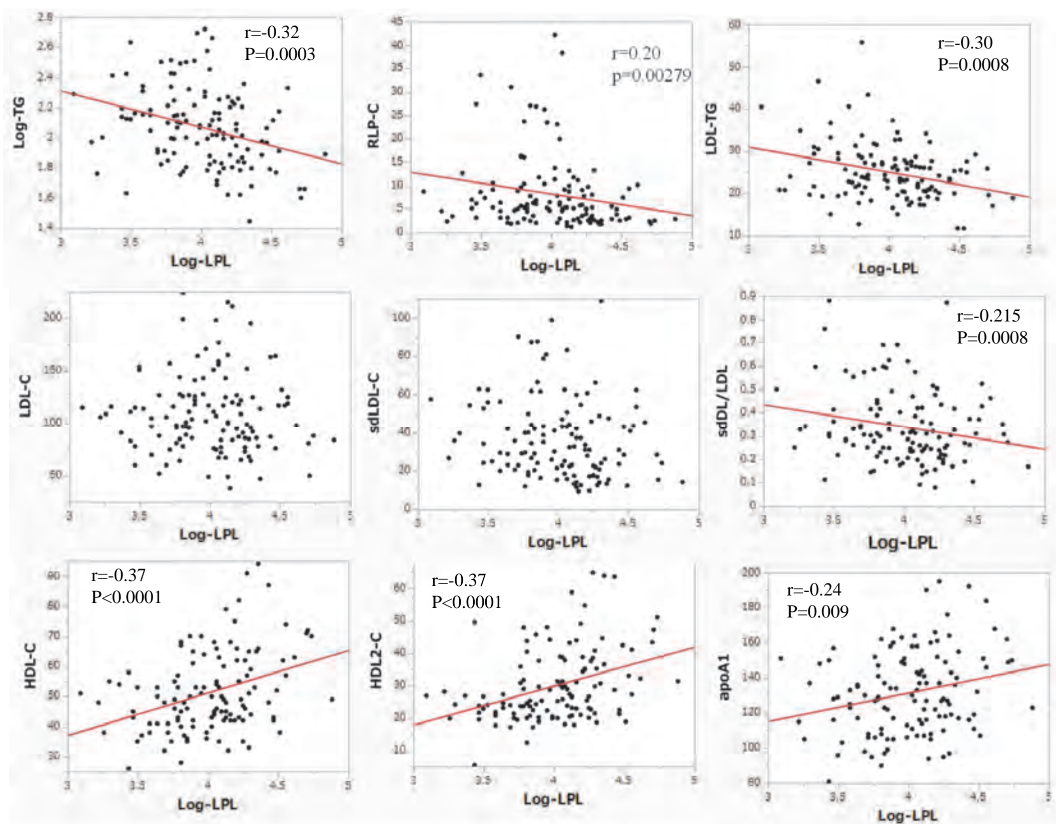

Figure 4 Relationship between log-LPL and various lipid parameters in type diabetic patients.

rin HTGL protein concentration and conventional and novel lipid parameters in patients with type 2 diabetes. Our study is the first to show pre-heparin HTGL levels in type 2 diabetes and their association with LDL and HDL subspecies. Pre-heparin HTGL levels in diabetics were higher than in healthy volunteers. However, the number of healthy controls was small and their age was young. Therefore, more controls of different ages need to fairly compare HTGL levels between diabetics and non-diabetics. The ELISA system used in this study had a strong positive correlation between pre-heparin HTGL concentration and PHP-HTGL concentration and a strong correlation between activity and concentration of HTGL in PHP [8]. Therefore, HTGL concentration may reflect HTGL activity even in pre-heparin plasma. We observed that pre-heparin HTGL concentrations positively cor-

related with LDL-C concentration. This is in good agreement with Glaser's report using pre-heparin HTGL activity [3]. HTGL facilitates the conversion from IDL to LDL by lipolyzing the TG moiety of IDL [1]. Therefore, the link between HTGL and LDL-C is reasonable. SdLDL-C and LDL-TG levels are expected to correlate significantly with HTGL as HTGL hydrolyzes TG-rich LDL and generalizes lipid-poor sdLDL particles [10,11]. Indeed, Muraba et al [21] reported that the HTGL concentration was positively correlated with sdLDL-C. Contrary to expectations, we could not find an intimate relationship between them. Nevertheless, there was an inverse relationship between HTGL and LDL-TG / LDL-C or LDL-TG / apoB. This is consistent with the action of HTGL on reducing TG in LDL particles.

HTGL, which has both TG lipase and phospholipase activity, plays an important role in phospholipid-enriched HDL metabolism. High HTGL activity reduces HDL2 and increases HDL3 [22]. HDL-C mainly consists of HDL2-C [20], thus HDL-C inversely correlate with PHP-HTGL activity [23]. Miyashita et al [7] reported that there was a weak relation of pre-heparin HTGL concentration against HDL-C. Unlike their results, we did not find any significant associations between pre-heparin HTGL and HDL-related measurements such as HDL2-C, HDL3-C, and apo AI (a main protein of HDL2).

We previously reported that both pre-heparin LPL and PHP-LPL were significantly lower in patients with type 2 diabetes, and there was a weak correlation between the pre-heparin LPL and PHP-LPL concentrations [15]. Our previous and current studies have consistently shown that pre-heparin LPL levels are inversely correlated with TG and positively correlated with HDL-C, which is well documented in PHP-LPL [23]. Moreover, we found an inverse association between pre-heparin LPL concentrations and RLP-C. LDL-TG, or sdLDL-C/LDL-C. This could be explained by LPL-induced TG-lowering action, because RLP-C, LDL-TG, and sdLDL-C/LDL-C are closely associated with TG. We also observed close relationship between pre-heparin LPL and HDL2-C or apo AI. LPL delivers unesterified cholesterol derived from the surface of TG-rich lipoproteins to smaller HDL particle via phospholipid transfer protein, resulting in generation of cholesterol-enriched HDL2 [24]. Our results suggest that the enzymatically inactive LPL still reflects the biological effect of this enzyme on all lipoprotein metabolism.

Nishimura et al [6] reported that HTGL protein concentrations in PHP in healthy subjects were about 2000 ng/mL. Pre-heparin HTGL levels were 57 ng/mL in diabetics and 44 ng/mL in healthy subjects, resulting in only 2-3% of PHP-HTGL levels. This absolute low concentration of pre-heparin HTGL may be the reason why the important physiological role of this enzyme in lipoprotein metabolism cannot be detected. On the other hand, PHP-LPL is about 250 ng/mL and pre-heparin LPL is 50 ng/mL, accounting for 20% of PHP-LPL [15]. The abundance of LPL in pre-heparin plasma could reflect physiological action of this enzyme. Several studies suggest an association between HTGL and central fatty obesity or fatty liver [1,8]. We failed to find this association, but found inverse correlation between LPL and VFA. Because pre-heparin LPL is reduced by metabolic disturbance [15], our results suggest that pre-heparin LPL is superior to HTGL for a marker of metabolic syndrome. However, majority of present subjects were treated with lipid-lowering and glucose-lowering drugs, which strongly affect lipid metabolism and obesity. Therefore, the effects of these drugs may mask the important effects of HTGL on lipoprotein metabolism or adiposity. This is the limit of our current research.

V. In conclusion

Pre-heparin plasma HTGL protein concentrations measured by a sensitive ELISA may have limited clinical relevance compared to pre-heparin plasma LPL protein at least in diabetic populations.

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COI

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Case Report

Cutaneous abscess with disseminated *Mycobacterium abscessus* from the lung

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ABSTRACT

A woman in her sixties presented to the dermatology department with erythematous nodules. Seventeen years earlier, she had been diagnosed with rheumatoid arthritis and treated with oral prednisolone. Three years later, she was also diagnosed with pulmonary non-tuberculous mycobacteriosis. Initially, we isolated *Mycobacterium intracellulare* from the bronchoscopy. However, 8 years later, the bacterial species changed to *Mycobacterium abscessus*. Skin biopsy was performed, and the culture was positive for *Mycobacterium abscessus*.

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Key Words
nontuberculous mycobacteriosis, *Mycobacterium abscessus*, cutaneous abscess

I. Introduction

The incidence of non-tuberculous mycobacteria (NTM) infections has been increasing in recent years. Among the NTM, *Mycobacterium intracellulare* (*M. intracellulare)* is a common cause of pulmonary infection. A majority of disseminated NTM infections occur in patients with a compromised immune status, such as infection with human immunodeficiency virus[1]. *M. abscessus*, a rapidly growing NTM, can cause skin infection. *M. abscessus* infection is resistant to numerous antibiotics and difficult to treat.

Herein, we describe a case of cutaneous abscess with disseminated *M. abscessus* from the lung.

II. Case report

A woman in her sixties presented to the dermatology department with erythematous nodules. The nodules were observed on bilateral legs 5 days after a follow-up computed tomography scan of the chest with contrast (**Figure 1**). Seventeen years earlier, she had been diagnosed with rheumatoid arthritis and treated with oral prednisolone (5 mg/day). Three years later, she was also diagnosed with pulmonary non-tuberculous mycobacteriosis. We initially isolated *M. intracellulare* from the bronchoscopy; however, 8 years later, the bacterial species changed to *M. abscessus*. Following the diagnosis of *M. intracellulare* infection, the patient was treated with clarithromycin, rifampicin, and ethambutol. A combination antibiotic therapy comprising oral clarithromycin, intravenous meropenem, and amikacin was initiated for the *M. abscessus* infection. However, pneumonia was refractory to the treatment. Three years prior to consultation, spinal fusion surgery was performed for an osteoporotic vertebral compression fracture. She was admitted to our hospital due to the presence of erythematous nodules

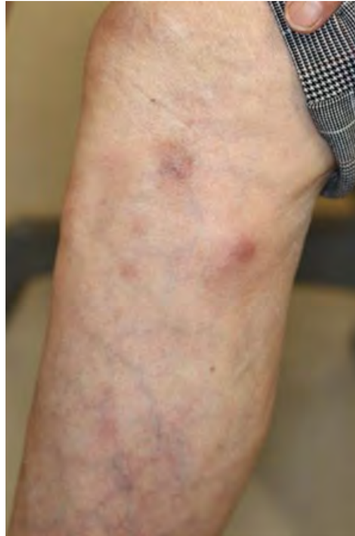

Figure 1 Appearance of the leg

and progressive pneumonia.

The laboratory data on admission were as follows: C-reactive protein 9.8 mg/dL; matrix metalloproteinase-3 187.4 ng/mL; and rheumatoid factor 244 IU/mL. Chest computed tomography revealed bilateral consolidation and reticular shadows (**Figure 2**).

The patient underwent a punch biopsy of her right leg; the culture was positive for rapidly growing mycobacteria (**Figure 3A**). Using DNA–DNA hybridization, the mycobacteria in clinical isolates were identified as *M. abscessus*. The minimum inhibitory concentrations were as follows: amikacin 16.0 μg/mL; kanamycin 16.0 μg/mL; clarithromycin >32.0 μg/mL; levofloxacin 32.0 μg/mL; streptomycin 32.0 μg/mL; rifampicin >32.0 μg/mL; ethambutol >128.0 μg/mL; ethionamide >16.0 μg/mL; and rifabutin 8.0 μg/mL. Histologically, extensive neutrophil infiltration with multinucleated Langhans giant cells throughout the dermis and the subcutaneous tissue was found (**Figure 3B**). However, there were no plump epithelioid cells detected. The results revealed non-caseating suppurative necrosis. Acid-fast bacilli were identified by Ziehl–Neelsen staining (**Figure 3C**).

III. Discussion

M. abscessus is a rapidly growing NTM and an environmental contaminant. It is regarded as chemotherapy-resistant NTM. *M. abscessus* can cause pulmonary and skin infections. In immunocompetent individuals, skin wounds are entry portals for *M. abscessus*. Disseminated infections, involving the lungs and skin, occur in immunocompromised patients. Guidelines established by the American Thoracic Society/Infectious Diseases Society of America (ATS/IDSA) recommend multidrug macrolide-based therapy based on susceptibility testing results[1].

Occasionally, pleural *M. abscessus* infection develops during the treatment of non-abscessus mycobacterial disease[2)-4)]. Culture examination should be considered in patients with NTM receiving long-term antibiotic therapy. A review of reported cutaneous *M. abscessus* cases showed various clinical characteristics[5]. Similar to our case, immunocompromised patients tend to have multiple lesions. *M. abscessus* has been associated with various foreign body infections[6]. In this case, metallic spinal implants may affect chronic infection with NTM. The ATS/IDSA recommended that surgery should generally be indicated for cases of *M. abscessus* infection with extensive disease, abscess formation, or where antibiotic therapy is difficult. Therefore, removal of foreign bodies is important and probably essential to recovery. In the present case, it was not possible to treat multiple lesions surgically or remove spinal implants, which may have increased the risk of chronic incurable infection.

Various types of bacteria and fungi cause granulomatous dermatitis. Because the treatment differs, the distinction of *M. abscessus* infection from other skin diseases is important. Combination antimicrobial therapy is recommended for disseminated M. abscessus infections. Differential diagnoses include cutaneous tuberculosis and swimming pool granuloma caused by *M. marinum*. *M. marinum* is an acid-fast bacillus, slightly larger than *M. tuberculosis*. *M. abscessus* is an elongated-shaped bacillus and, as the name implies, tends to from soft tissue abscesses. Sufficient clinical information is required for accurate pathological diagnosis.

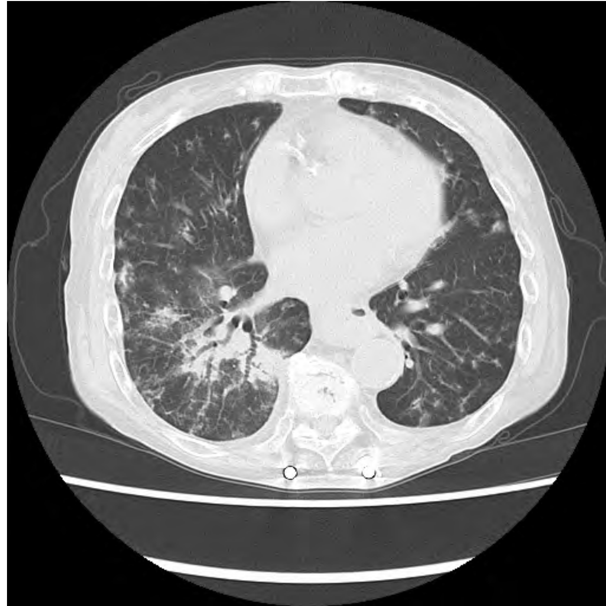

Figure 2 Chest computed tomography on admission showing bilateral consolidation and reticular shadows. Metallic spinal implants are visible.

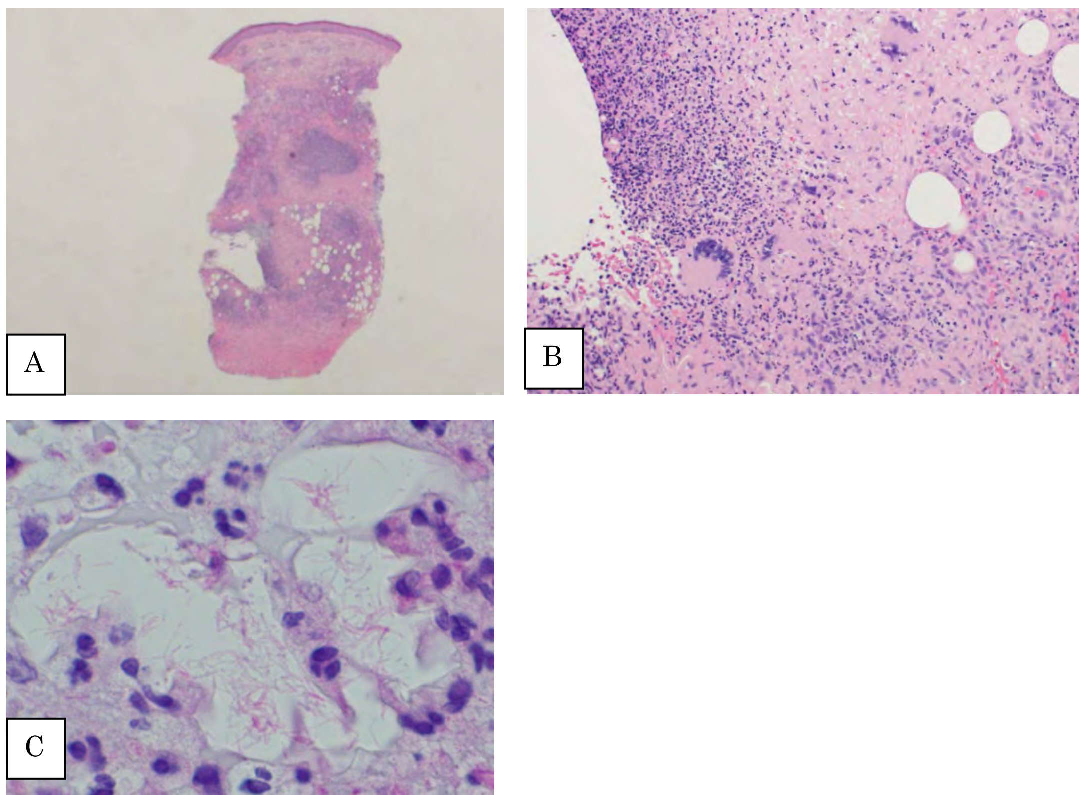

Figure 3
A) A low-power view of the skin (× 1.25)
B) Abscess with multinucleated giant cells (× 20)
C) Acid-fast bacilli shown by Ziehl–Neelsen staining (× 100).

Conflict of interest

The authors declare that they have no conflicts of interest.

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