*Editorial*

# Analytical Quality Control in Physiological Function Testing in the era of Artificial Intelligence

*Tatsuo Shimosawa, MD, PhD**

* Department of Clinical Laboratory, School of Medicine, International University of Health and Welfare, Chiba, Japan.
E-mail: Shimosawa-lab@ihwg.jp

〔Lab Med Int 2025; 4(4): 103-104〕

**See article volume 4(4): 114-124**

Physiological function tests such as electrocardiography (ECG), ultrasound, and pulmonary function assessments are critical for diagnosing cardiovascular, respiratory, and systemic conditions. Analytical quality control ensures that these tests produce accurate and reproducible results[1]. In the era of artificial intelligence (AI), Quality control (QC) control has two important meaning. One is AI can be used in maintaining robust QC systems is vital to prevent diagnostic errors and optimize patient care. The other is without well quality controlled data, AI can not learn from the data to draw correct and robust interpretations.

## Analytical QC in ECG Testing

Electrocardiography relies on precise signal acquisition and interpretation. Analytical QC involves calibration of ECG machines, verification of signal fidelity, and artifact detection. Common analytical errors include baseline wander, electrode misplacement, and noise interference. AI algorithms now assist in detecting subtle waveform anomalies and validating automated interpretations. In the current issue of Laboratory Medicine International (LMI), Ogasawara and her colleagues reported survey of the quality management of ECG in ISO15189 accredited clinical laboratory in Japan[2]. Their survey shows surprisingly wide fluctuations of values and certain number of laboratories do not meet the satisfactory internal or external accuracy control level.

## AI Integration in Analytical QC[3]

AI enhances QC by enabling predictive maintenance, anomaly detection, and adaptive QC protocols. For ECG, AI can identify electrode misplacement patterns; for ultrasound, it can evaluate image quality metrics; for pulmonary function tests (PFTs), it can predict calibration drift. Challenges include data standardization, algorithm transparency, and regulatory compliance.

## AI Integration in clinical decision making[3]

It is not needed to stress that imaging, such as ECG or ultrasound are very important in our clinical evaluation. Numerical evaluation of ECG as well as the shape of each component of wave, and, ultrasound images all over the body plays a critical and pivotal role in clinical diagnosis these days. After emerging of AI, AI-powered ECG or ultrasound is becoming more mature and getting closer to routine clinical applications in recent times because of an increased need for efficient and objective acquisition and evaluation of ECG and ultrasound images. AI-assisted image acquisition made us possible to assess image quality in real time and prompt diagnosis. Because ultrasound images involve operator-, patient-, and scanner-dependent variations, the adaptation of classical machine learning methods to clinical applications becomes challenging. With their self-learning ability, deep-learning (DL) methods are able to harness exponentially growing graphics processing unit computing power to identify abstract and complex imaging features. To achieve high QCquality control in scanner and data acquisition system is essential to obtain powerful AI-assisted laboratory examination[4].

## Future Directions

Future QC frameworks will integrate AI-driven analytics with traditional QC principles. Laboratories and diagnostic centers must invest in robust data governance, staff training, and collaborative research to validate AI

tools. Harmonization of QC standards across physiological testing modalities will ensure consistency and reliability globally.

## Conclusion

Analytical QC in physiological function testing is indispensable for accurate diagnosis and patient safety. As AI reshapes diagnostic workflows, maintaining rigorous QC systems for ECG, ultrasound, and PFTs will be essential to realize the full potential of technological innovation.

### References

1) ISO 15189: Medical laboratories – Requirements for quality and competence. International Organization for Standardization; 2022.
2) Ogasawa N, Tanabe A, Masaki K, Asashi K, Mochida T, Daimon M, Yokoyama N, Tojo N, Shirota Y, Furukawa T. Current status of quality management of standard 12-leads electrocardiography at ISO 15189 accredited medical institutes in Japan. LMI. 2025; 4 (4) : 114-24.
3) Topol EJ. High-performance medicine: AI in diagnostics. Nat Med. 2019; 25 (1) : 44-56.
4) Wiens J, Saria S, Sendak M, Ghassemi M, Liu VX, Doshi-Velez F, Jung K, Heller K, Kale D, Saeed M, Ossorio PN, Thadaney-Israni S, Goldenberg A. Do no harm: a roadmap for responsible machine learning for health care. Nat Med. 2019 Sep; 25 (9) : 1337-40.

*Original*

# Associations between inflammatory markers and all-cause mortality in the general population: the Nagahama study.

*Aya Shoji-Asahina*[*1], *Kazuya Setoh*[*2], *Takahisa Kawaguchi*[*3], *Takeo Nakayama*[*1, 4],
*Fumihiko Matsuda*[*3], †*Yasuharu Tabara*[*1, 3] *and the Nagahama Study Group*

†Correspondence: Graduate School of Public Health, Shizuoka Graduate University of Public Health Kita-ando 4-27-2, Aoi-ku, Shizuoka 420-0881, Japan
Tel: +81-54-295-5400; Fax: +81-54-248-3520;
E-mail: tabara@s-sph.ac.jp
Received July 9, 2025; accepted July 28, 2025
*1 Graduate School of Public Health, Shizuoka Graduate University of Public Health, Shizuoka 420-8527, Japan
*2 Department of Epidemiology for Community Health and Medicine, Kyoto Prefectural University of Medicine, Kyoto 602-8566, Japan
*3 Center for Genomic Medicine, Kyoto University Graduate School of Medicine, Kyoto 606-8507, Japan
*4 Department of Health Informatics, Kyoto University School of Public Health, Kyoto 606-8501, Japan

## ABSTRACT

**Background:** Inflammatory markers, especially C-reactive protein (CRP), have been reported to be associated with all-cause mortality. In addition, α1-antitrypsin and white blood cell (WBC)-based inflammatory markers were suggested to represent mortality risk. We aimed to investigate whether simultaneous assessment of these markers was more useful for evaluating mortality risk than compared to individual assessment.
**Methods:** This longitudinal study included 5,970 Japanese community residents (mean age 62.9 years). Circulating levels of inflammatory markers were measured from baseline blood samples. All-cause mortality was ascertained by referring to the residential records.
**Results:** During a mean follow-up duration of 13.5 years, 550 deaths occurred. Kaplan–Meier curves for mortality showed significant differences across the quintiles of each marker (log-rank test: $P < 0.05$). Results of the Cox proportional hazard model adjusted for potential covariates indicated that α 1-antitrypsin (fifth quintile: hazard ratio 1.73, $P < 0.001$) and CRP (fourth quintile: hazard ratio 1.49, $P = 0.001$; fifth quintile: hazard ratio 1.33, $P = 0.068$) were significantly associated with mortality. Among the WBC-based markers, platelet-to-lymphocyte ratio (hazard ratio 1.38, $P = 0.002$), systemic immune–inflammation index (hazard ratio 1.27, $P = 0.014$), and lymphocyte-to-monocyte ratio (hazard ratio 1.39, $P = 0.035$) showed significant associations. When these markers were included in the same model, α1-antitrypsin, but not CRP, showed pronounced association, whereas the WBC-based markers showed significant but weak associations.
**Conclusions:** α1-antitrypsin was identified as a good marker for long-term mortality risk assessment in the general population. Combining these markers might help identify high risk populations.
〔Lab Med Int 2025; 4(4): 105-113〕

**Key Words**
white blood cell count, α1-antitrypsin, C-reactive protein, all-cause mortality, general population

## I. Introduction

Systemic inflammation is exaggerated in patients with cardiovascular diseases [1)2)] and cancers [2)]. Among several peripheral blood markers for systemic inflammation, high-sensitivity C-reactive protein (hsCRP) is a well-investigated marker for indicating increased risk of cardiovascular [3)] and all-cause mortalities [4)-7)]. hsCRP

is an acute-phase reactant, the production of which is stimulated by interleukin-6 released from activated immune cells [1]. A previous report revealed that hsCRP was associated with all-cause mortality in a general Japanese population aged 50 years or over [8]. α1-antitrypsin (AAT), another acute-phase inflammatory marker, has also been reported to be associated with cardiovascular disease events [9)-13)] and all-cause mortality [8] in general populations. AAT is a major serine protease inhibitor with broad-spectrum anti-inflammatory, immunomodulatory, and anti-infective tissue-repair functions [14]. In addition, several markers based on white blood cell (WBC) counts, such as neutrophil-to-lymphocyte ratio (NLR), have been suggested to indicate systemic inflammation and reported to be associated with the prognosis of certain cancers [15)-16)], sepsis [18], and stroke [19]. NLR has also been reported to be associated with the severity of coronary artery disease patients [20]. Other WBC-based markers, including platelet-to-lymphocyte ratio (PLR), systemic immune–inflammation index (SII), and lymphocyte-to-monocyte ratio (LMR), have also been suggested to be associated with a worse prognosis in cancer patients [21)-23)]. Even in a general population, NLR, PLR, SII, and LMR were suggested to be associated with all-cause mortality [24)-28)].

Therefore, we hypothesized that the simultaneous assessment of conventional and WBC-based inflammatory markers may be more useful for mortality risk evaluation than individual assessment. Furthermore, previous findings on WBC-based markers have been focused on Western populations [24)-26)28)], with limited results in Asian populations [27)29)]. To our knowledge, no reports have been published on Japanese cohorts. We aimed to investigate this hypothesis by the analysis of large-scale longitudinal study of a general Japanese population.

## II. Methods

### *Study population*

We analyzed the data of the Nagahama Study [8)30)], an ongoing longitudinal study based on community residents of Nagahama City, Japan, located in central Japan with approximately 113,000 inhabitants in 2024. Participants of the Nagahama Study were recruited at a baseline survey performed between 2008 and 2010. Nagahama City residents aged 30–86 years who were living independently without physical impairment or dysfunction were eligible to participate. Of the baseline population (N = 9,764), 5,970 were ultimately included in this study after excluding participants who met the following exclusion criteria; younger than 50 years (N = 3,736), using hemodialysis therapy (N = 4), pacemaker implantation (N = 11), having clinical values widely deviated from their distributions [platelet count ≥ 550 × $10^9$/L (N = 1), NLR ≥ 9 (N = 5), gamma-glutamyl transferase (γGT) ≥ 500 IU/L (N = 10), ALT ≥ 200 IU/L (N = 2)], and incomplete measurement of required clinical values (N = 25).

All procedures were approved by the Ethics Committee of Kyoto University Graduate School of Medicine and the Nagahama Municipal Review Board. Written informed consent was obtained from all participants.

### *All-cause mortality*

All-cause mortality was identified by reviewing residential registry records managed by the Nagahama City Office. Participants who had relocated out of Nagahama City were censored. Follow-up period was calculated from participation in the baseline survey to the date of relocation, death or to current end of the follow-up period (March 31, 2024).

### *Inflammatory markers*

Serum levels of AAT and hsCRP were measured using a blood sample drawn at baseline in a commercial laboratory (SRL Inc., Tokyo, Japan) using the N-antiserum to Human Alpha-1-Antitrypsin Kit or N-Latex CRP II Kit (Siemens Healthcare Diagnostics, Munich, Germany). Other blood markers were measured using the same sera in another commercial laboratory (Medic Inc., Shiga, Japan). Blood cell counts and blood cell fractions were measured using an automated hematology analyzer (Sysmex XE–2100, Sysmex Corporation, Kobe, Japan) using the blood specimens drawn at baseline. NLR, PLR, SII, and LMR were calculated using the following formulas:

NLR = neutrophil count/lymphocyte count

PLR = platelet count/lymphocyte count

SII = (neutrophil count × platelet count)/lymphocyte count

LMR = lymphocyte count/monocyte count

### *Basic clinical parameters*

Other clinical parameters used in this study were obtained at baseline. Data on histories of cancers and cardiovascular diseases, medication use, and smoking and drinking habits were obtained using a structured questionnaire. Heavy drinking was defined as consuming ≥ 2 Go (men) or ≥ 1 Go (women) of alcohol per sitting. Go is a Japanese traditional liquor unit that corresponds to 22 g of ethanol. Blood pressure was measured twice after a few minutes of rest in a sitting position using a cuff-oscillometric device (HEM-9000AI; Omron Healthcare,

Kyoto, Japan). The mean of two readings was used as the representative value.

### *Statistical analysis*

Values were expressed as means ± standard deviations, medians and interquartile ranges, or frequencies. The Student's t-test, analysis of variances, Mann–Whitney U test and Kruskal–Wallis test were used to assess group differences in numerical variables, whereas chi-squared tests were used to assess frequency differences. Spearman's rank correlation coefficient was used to examine correlations among numerical variables. Mortality rate was calculated per 10,000 person-years. Survival curves across quintiles of inflammatory markers were depicted using the Kaplan–Meier method, and group differences in the survival curves were assessed using the log-rank test.

We first used Cox proportional hazards models to identify factors independently associated with all-cause mortality. Each model included quintiles of one WBC-based marker and was adjusted for standard covariates, including age, sex, body mass index, current smoking, heavy drinking, history of cancer, history of cardiovascular disease, mean blood pressure, hemoglobin A1c, high-density lipoprotein cholesterol, low-density lipo-

**Table 1** Baseline clinical characteristics of the study participants (N = 5,970)

| | Alive N = 5,420 | Died N = 550 | P |
|---|---|---|---|
| Age, years old | 62.4 ± 6.3 | 67.1 ± 5.6 | <0.001 |
| Sex male % | 32.9 | 60.4 | <0.001 |
| Body mass index, kg/m2 | 22.7 ± 3.0 | 22.8 ± 3.2 | 0.475 |
| Current smoking, % | 10.2 | 19.6 | < 0.001 |
| Heavy drinking, % | 6.4 | 8.7 | 0.038 |
| History of cancers, % | 5.8 | 10.6 | < 0.001 |
| History of cardiovascular diseases, % | 3.6 | 9.1 | < 0.001 |
| Systolic blood pressure, mmHg | 128 ± 17 | 132 ± 18 | < 0.001 |
| Diastolic blood pressure, mmHg | 78 ± 11 | 79 ± 11 | < 0.001 |
| ***Plasma markers*** | | | |
| Hemoglobin A1c, % | 5.6 ± 0.5 | 5.7 ± 0.8 | < 0.001 |
| High-density lipoprotein cholesterol, mg/dL | 65 ± 17 | 60 ± 17 | < 0.001 |
| Low-density lipoprotein cholesterol, mg/dL | 129 ± 30 | 122 ± 32 | < 0.001 |
| Albumin, g/dL | 4.4 ± 0.2 | 4.4 ± 0.2 | < 0.001 |
| Creatinine, mg/dL | 0.7 [0.6–0.8] | 0.8 [0.6–0.9] | < 0.001 |
| Alanine aminotransferase, IU/L | 19 [15–25] | 19 [15–26] | 0.908 |
| Gamma-glutamyl transferase, IU/L | 23 [16–36] | 27 [19–43] | < 0.001 |
| High-sensitivity C-reactive protein, μ g/mL | 0.34 [0.17–0.69] | 0.47 [0.22–0.93] | < 0.001 |
| α1-antitrypsin, mg/dL | 131 ± 18 | 140 ± 23 | < 0.001 |
| ***Blood cell counts*** | | | |
| White blood cells, × $10^9$ /L | 5.8 ± 1.4 | 6.1 ± 1.7 | < 0.001 |
| Neutrophils, × $10^9$ /L | 3.3 ± 1.0 | 3.5 ± 1.2 | < 0.001 |
| Lymphocytes, × $10^9$ /L | 2.0 ± 0.6 | 2.1 ± 0.7 | 0.840 |
| Monocytes, × $10^9$ /L | 0.3 ± 0.1 | 0.3 ± 0.1 | < 0.001 |
| Platelet counts, × $10^9$ /L | 226 ± 49 | 223 ± 57 | 0.194 |
| ***White blood cell-based inflammatory markers*** | | | |
| NLR | 1.68 ± 0.72 | 1.86 ± 0.85 | < 0.001 |
| PLR | 117 ± 38 | 118 ± 48 | 0.485 |
| SII | 381 ± 189 | 418 ± 240 | < 0.001 |
| LMR | 7.93 ± 2.84 | 6.99 ± 2.62 | < 0.001 |

Values are mean ± standard deviation, median and interquartile range, or frequency. Statistical significance was assessed by the analysis of variance or the Chi-squared test. Cardiovascular diseases include symptomatic stroke, angina pectoris, and myocardial infarction. NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; SII, systemic immune–inflammation index; LMR, lymphocyte-to-monocyte ratio.

protein cholesterol, creatinine, albumin, alanine aminotransferase, and gamma-glutamyl transferase. Based on the results of these initial analyses, we then conducted Cox proportional hazards models that included the most abnormal quintiles of the statistically significant WBC-based markers, in order to evaluate their associations after further adjustment for serum inflammatory markers. The inflammatory markers included in this analysis were the fifth quintile (Q5) of AAT and a high hsCRP group, defined as either the fourth (Q4) or fifth (Q5) quintile. Finally, to provide clinically interpretable results, we conducted additional Cox proportional hazards analyses combining the high levels of these statistically significant inflammatory and WBC-based markers without adjusting for other covariates, in order to calculate crude hazard ratios. To account for multicollinearity, each WBC-based marker was included in the model separately. The results are presented as hazard ratios (HRs) with 95% confidence intervals (CIs).

Statistical analyses were primarily conducted using JMP software, version 17.0.0 (SAS Institute, Cary, NC, USA). In addition, restricted cubic spline analyses using the Cox proportional hazards model were performed in R software, version 4.5.1, with the survival and rms packages. P-values $<0.05$ were considered significant.

## III. Results

The mean age of the study participants was 62.9 ± 6.4 years, and 35.4% were men. During a mean follow-up duration of 13.5 years, 550 deaths were observed. Supplementary Figure 1 illustrates cubic splines showing the association between baseline WBC-based markers or serum inflammatory markers and the HRs for all-cause mortality. The baseline clinical characteristics of the study participants are summarized in **Table 1** separately for cases of death and survival. The death group was older, included more men and smokers, and had a higher prevalence of cancer and cardiovascular disease at baseline. hsCRP, AAT, NLR and SII were significantly higher, and LMR was significantly lower in the death group. Clinical characteristics of study populations in studies that investigated the prognostic significance of WBC-based markers are summarized in Supplementary Table 1. Our study population had a relatively small body size, a low frequency of smokers and patients with diabetes, and a low mortality rate. In addition, levels of WBC-based markers in this population were better than those in other populations.

**Table 2** shows mortality rates by the quintiles of each inflammatory marker. Differences in the survival curves among the quintiles (**Figure 1**) were statistically significant for all markers. Because the clinical characteristics differed significantly among the quintiles (Supplementary **Tables 2-7**), a covariate-adjusted Cox proportional hazards model analysis was performed to identify markers independently associated with all-cause mortality (**Table 3**). AAT showed marked association with mortality even when individuals with circulating AAT levels less than 100 mg/dL (n = 130), possible cases of congenital AAT deficiency, were excluded from the analysis (Supplementary **Table 8**). Other markers, namely PLR, SII, LMR, and hsCRP, but not NLR, were also associated with mortality, although the significance was relatively weak. When the conventional inflammatory markers and one of the significant WBC-based inflammatory markers were included in the same model, PLR and SII were independently associated with all-cause mortality along with AAT (PLR [Q5] HR = 1.38, 95% CI: 1.13–1.69, P = 0.002, SII [Q5] HR = 1.27, 95% CI: 1.05–1.54. P = 0.014) (**Table 4**). hsCRP did not show significant association in any model. **Figure 2** shows crude HR for all-cause mortality by the combination of AAT and PLR or SII, indicating a stepwise association between the combined markers and all-cause mortality. For the combination of PLR and AAT, the highest HR was observed in the group with both high PLR and high AAT (HR = 2.74, 95% CI: 2.05–3.67, P < 0.001), followed by the group with high AAT only (HR = 2.20, 95% CI: 1.80–2.69, P < 0.001), and the group with high PLR only (HR = 1.17, 95% CI: 0.91–1.50, P = 0.232), using the group with neither high PLR nor high AAT as the reference. Similarly, for the combination of SII and AAT, the highest HR was observed in the group with both high SII and high AAT (HR = 2.89, 95% CI: 2.23–3.76, P < 0.001), followed by the group with high AAT only (HR = 2.23, 95% CI: 1.81–2.76, P < 0.001), and the group with high SII only (HR = 1.46, 95% CI: 1.14–1.87, P = 0.003), using the group with neither high SII nor high AAT as the reference.

Supplementary **Figure 2** shows changes in the adjusted HR of each marker with increasing follow-up duration. The HR of AAT was consistently significant, whereas those of other markers decreased gradually, indicating that the prognostic significance of each marker varied with follow-up duration.

## IV. Discussion

In this longitudinal study of a large general population, we showed that conventional inflammatory markers (such as AAT) and WBC-based markers (such as PLR and SII) were independently associated with all-cause mortality. The HRs increased linearly with the combination of these

**Table 2** Summary statistics of mortality rate by inflammatory marker quintile

| | Quintiles | | | | |
|---|---|---|---|---|---|
| ***NLR*** | Q1 | Q2 | Q3 | Q4 | Q5 |
| Person-years | 16,194 | 16,155 | 16,108 | 16,140 | 15,988 |
| All-cause mortality | 84 | 104 | 103 | 107 | 152 |
| Mortality rate | 51.9 | 64.4 | 63.9 | 66.3 | 95.1 |
| ***PLR*** | | | | | |
| Person-years | 15,982 | 16,176 | 16,241 | 16,188 | 16,011 |
| All-cause mortality | 134 | 103 | 95 | 88 | 130 |
| Mortality rate | 83.8 | 63.7 | 58.5 | 54.4 | 81.2 |
| ***SII*** | | | | | |
| Person-years | 16,098 | 16,210 | 16,210 | 16,121 | 15,958 |
| All-cause mortality | 107 | 97 | 92 | 101 | 153 |
| Mortality rate | 66.5 | 59.8 | 56.8 | 62.7 | 95.9 |
| ***LMR*** | | | | | |
| Person-years | 15,902 | 16,165 | 16,067 | 16,246 | 16,216 |
| All-cause mortality | 169 | 121 | 110 | 88 | 62 |
| Mortality rate | 106.3 | 74.9 | 68.5 | 54.2 | 38.2 |
| ***hsCRP*** | | | | | |
| Person-years | 16,255 | 16,100 | 16,306 | 16,015 | 15,920 |
| All-cause mortality | 72 | 95 | 100 | 134 | 149 |
| Mortality rate | 44.3 | 59.0 | 61.3 | 83.7 | 93.6 |
| ***AAT*** | | | | | |
| Person-years | 15,199 | 16,276 | 15,533 | 17,661 | 15,929 |
| All-cause mortality | 72 | 77 | 87 | 118 | 196 |
| Mortality rate | 47.4 | 47.3 | 56.0 | 66.8 | 123.0 |

The mortality rate is shown per 10,000 person-years.
NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; SII, systemic immune–inflammation index; LMR, lymphocyte-to-monocyte ratio; hsCRP, high-sensitivity C-reactive protein; AAT, α1-antitrypsin.

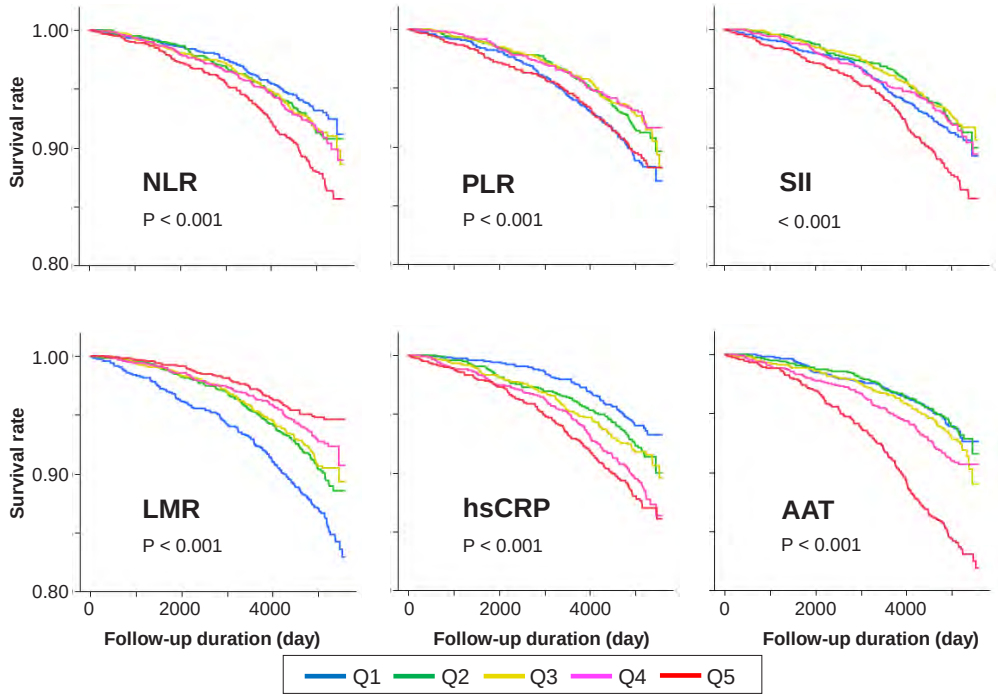


**Figure 1** Kaplan-Meier curve for all-cause mortality. Statistical significance was assessed by log-rank test. NLR: neutrophil-to-lymphocyte ratio, PLR: platelet-to-lymphocyte ratio, SII: systemic immune-inflammation index, LMR: lymphocyte-to-monocyte ratio; hsCRP, high-sensitivity C-reactive protein; AAT, α 1-antitrypsin.

**Table 3** Cox proportional hazards model analysis for all-cause mortality across quintiles of inflammatory markers (N = 5,970)

| | | HR (95% CI) | P |
|---|---|---|---|
| ***NLR*** | Q1 | Reference | |
| | Q2 | 1.06 (0.80–1.42) | 0.686 |
| | Q3 | 1.04 (0.78–1.39) | 0.782 |
| | Q4 | 1.01 (0.75–1.34) | 0.967 |
| | Q5 | 1.28 (0.97–1.67) | 0.078 |
| ***PLR*** | Q1 | Reference | |
| | Q2 | 0.92 (0.71–1.20) | 0.551 |
| | Q3 | 0.86 (0.66–1.13) | 0.284 |
| | Q4 | 0.86 (0.65–1.13) | 0.284 |
| | Q5 | 1.32 (1.03–1.70) | 0.031 |
| ***SII*** | Q1 | Reference | |
| | Q2 | 0.90 (0.69–1.19) | 0.478 |
| | Q3 | 0.87 (0.66–1.15) | 0.324 |
| | Q4 | 0.95 (0.72–1.25) | 0.716 |
| | Q5 | 1.29 (1.00–1.65) | 0.049 |
| ***LMR*** | Q1 | 1.39 (1.02–1.90) | 0.035 |
| | Q2 | 1.18 (0.86–1.62) | 0.296 |
| | Q3 | 1.27 (0.93–1.75) | 0.134 |
| | Q4 | 1.20 (0.86–1.66) | 0.284 |
| | Q5 | Reference | |
| ***hsCRP*** | Q1 | Reference | |
| | Q2 | 1.22 (0.89–1.66) | 0.210 |
| | Q3 | 1.19 (0.87–1.62) | 0.285 |
| | Q4 | 1.49 (1.10–2.01) | 0.001 |
| | Q5 | 1.33 (0.98–1.80) | 0.068 |
| ***AAT*** | Q1 | Reference | |
| | Q2 | 0.94 (0.68–1.30) | 0.710 |
| | Q3 | 1.09 (0.79–1.49) | 0.602 |
| | Q4 | 1.08 (0.80–1.46) | 0.599 |
| | Q5 | 1.73 (1.31–2.29) | < 0.001 |

Adjusted standard factors were age, sex, body mass index, current smoking, heavy drinking, history of cancer, history of cardiovascular disease, mean blood pressure, hemoglobin A1c, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, creatinine, albumin, alanine aminotransferase, and gamma-glutamyl transferase.
HR; hazard ratio; CI, confidence interval; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; SII, systemic immune–inflammation index, LMR, lymphocyte-to-monocyte ratio; hsCRP, high-sensitivity C-reactive protein; AAT, α1-antitrypsin.

**Table 4** Cox proportional hazards model analysis of all-cause mortality (N = 5,970)

| | | HR (95% CI) | P |
|---|---|---|---|
| Model 1 | PLR (Q5) | 1.38 (1.13–1.69) | 0.002 |
| | hsCRP (Q4 or Q5) | 1.09 (0.90–1.31) | 0.388 |
| | AAT (Q5) | 1.61 (1.34–1.94) | <0.001 |
| Model 2 | SII (Q5) | 1.27 (1.05–1.54) | 0.014 |
| | hsCRP (Q4 or Q5) | 1.08 (0.89–1.30) | 0.440 |
| | AAT (Q5) | 1.59 (1.32–1.92) | <0.001 |
| Model 3 | LMR (Q1) | 1.23 (0.93–1.36) | 0.230 |
| | hsCRP (Q4 or Q5) | 1.09 (0.91–1.32) | 0.339 |
| | AAT (Q5) | 1.63 (1.35–1.96) | <0.001 |

Adjusted factors were age, sex, body mass index, current smoking, heavy drinking, history of cancer, history of cardiovascular disease, mean blood pressure, hemoglobin A1c, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, creatinine, albumin, alanine aminotransferase, gamma-glutamyl transferase and AAT.
HR; hazard ratio; CI, confidence interval; NLR, neutrophil-to-lymphocyte ratio; SII, systemic immune–inflammation index; hsCRP, high-sensitivity C-reactive protein; AAT, α 1-antitrypsin.

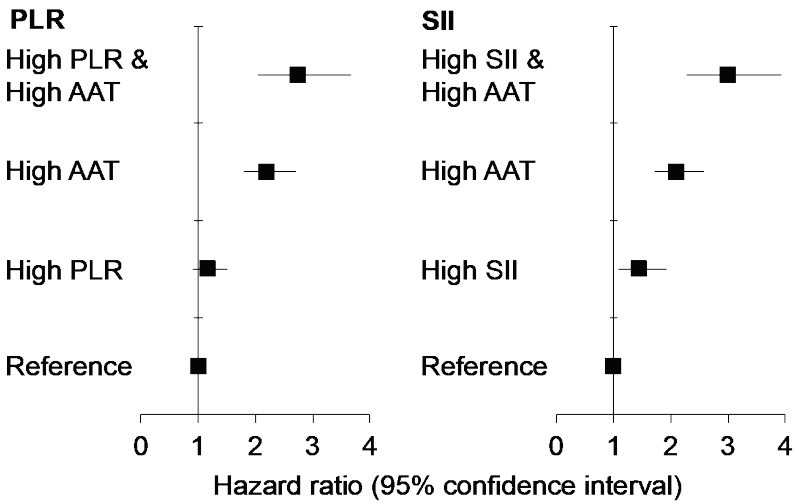


**Figure 2** Crude hazard ratio for all-cause mortality.
High α 1-antitrypsin (AAT): ≥ 146 mg/dL; high platelet-to-lymphocyte ratio (PLR): ≥ 144; high systemic immune-inflammation index (SII): ≥ 495.

markers.

PLR and SII were significantly associated with all-cause mortality. However, their HRs in the combination analysis with AAT were substantially smaller than that of AAT, which did not support PLR and SII use in population health practice, despite their ease of measurement. The lack of or weak associations was possibly due to our study population being relatively healthy, with low BMI, less frequent smoking and diabetes, and low mortality rates, compared with previous studies that reported the prognostic significance of WBC-based markers. In addition, NLR, PLR, and SII levels were lower in our study population. Although it is difficult to directly compare the results of our study with those of previous studies owing to large population differences including ethnicity, WBC-based markers may be useful in populations with more pronounced systemic inflammations.

In our previous analysis of the Nagahama study population, we found that AAT and hsCRP were independently associated with all-cause mortality [8]. However, in the current analysis of the same population with approximately 4 years of extended follow-up period, the HRs of hsCRP did not reach statistical significance when hsCRP and AAT were included in the same model. A reason for the discrepancy may lie in the gradual decreases in the HR of hsCRP in proportion to follow-up duration. The HRs of PLR, SII, and LMR also showed gradual decline, and a similar trend was observed in the analysis of NLR in the Rotterdam study [24] and the National Health and Nutrition Examination Survey [25], indicating that hsCRP and WBC-based markers may be useful in predicting relatively short-term prognosis. In contrast, AAT was associated with all-cause mortality in a study with a follow-up of more than 10 years probably due to AAT indicating long-term persistent low-level inflammation [31].

Several studies on the prognostic significance of WBC-based markers did not include CRP in the model, in order to examine the usefulness of WBC-based markers as a proxy for CRP [15,22,23,25,27-29]. In our study, WBC-based markers did not show clear associations with mortality even when hsCRP and AAT were not included in the model, indicating a limited usability of WBC-based markers. In settings where it is easily measurable, such as Japan, CRP is preferred for the assessment of potential inflammation in a general population. Our results strongly suggest that AAT should also be emphasized as an inflammatory marker in addition to CRP.

A strength of this study was the large sample size and availability of various clinical measures, which allowed for a comparison of the prognostic significance of WBC-based and conventional inflammatory markers. However, several study limitations should be considered with caution in interpreting the results. First, we did not consider cause of death owing to the limited number of deaths. Given the results of previous studies, WBC-based markers may be closely associated with cancer and cardiovascular mortalities. Second, WBC differential counts were measured using an automated hematology analyzer, whereas the visual method is considered as an objective standard. However, any discrepancies in the counts due to the differences in measurement methods were small. The use of automated hematology analyzers may not significantly affect the present results.

In conclusion, AAT was identified as a good marker for long-term mortality risk assessment in the general population. Some of WBC-based inflammatory markers were

associated with mortality, although the prognostic significance was limited when WBC-based inflammatory markers were considered individually. However, combining these markers with AAT might be useful for identifying populations at higher risk.

**Author Contributions**

**AS:** Conceptualization, Methodology, Formal analysis, Writing - Original Draft. **KS:** Supervision, Investigation, Data Curation. **TK:** Supervision, Investigation, Data Curation. **TN:** Supervision, Project administration, Funding acquisition. **FM:** Supervision, Project administration, Funding acquisition. **YT:** Conceptualization, Methodology, Investigation, Resources, Data Curation, Supervision, Project administration, Funding acquisition, Writing – review & editing.

**Acknowledgements**

We would like to thank all members of the Nagahama study group for their assistance in conducting the Nagahama study. We are very grateful to the Nagahama City Office and the non-profit organization, Zeroji Club, for their assistance in the Nagahama study. We would like to thank Editage (www.editage.jp) for English language editing.

**Conflict of Interest**

The authors have no conflicts of interest directly relevant to the content of this article.

**Financial Support**

The work was supported by a university grant, the Center of Innovation Program, the Global University Project from the Ministry of Education, Culture, Sports, Science and Technology of Japan (25293141, 26670313, 25253059, 26293198, 17H04182, 17H04126, 18K18450, 17H04123, 21H04850); the Practical Research Project for Rare/Intractable Diseases (ek0109070, ek0109283, ek0109196, ek0109348), the Program for an Integrated Database of Clinical and Genomic Information (kk0205008), the Research and Development Grants for Dementia (dk0207006, dk0207027), the Practical Research Project for Lifestyle-related Diseases including Cardiovascular Diseases and Diabetes Mellitus (ek0210066, ek0210096, ek0210116), the Research Program for Health Behavior Modification by Utilizing IoT (le0110005, le0110013), the Research and Development Grants for Longevity Science (dk0110040) from the Japan Agency for Medical Research and Development (AMED), Takeda Medical Research Foundation, Mitsubishi Foundation, Daiwa Securities Health Foundation, and Sumitomo Foundation.

**References**

1) Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med. 2005; 352(16):1685-95. doi: 10.1056/NEJMra043430.
2) Furman D, Campisi J, Verdin E, et al. Chronic inflammation in the etiology of disease across the life span. Nat Med. 2019; 25(12): 1822-32. doi: 10.1038/s41591-019-0675-0.
3) Kaptoge S, Di Angelantonio E, Lowe G, et al. C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. Lancet. 2010; 375(9709): 132-40. doi: 10.1016/S0140-6736(09)61717-7.
4) Zacho J, Tybjaerg-Hansen A, Nordestgaard BG. C-reactive protein and all-cause mortality--the Copenhagen City Heart Study. Eur Heart J. 2010; 31(13): 1624-32. doi: 10.1093/eurheartj/ehq103.
5) Li Y, Zhong X, Cheng G, et al. Hs-CRP and all-cause, cardiovascular, and cancer mortality risk: A meta-analysis. Atherosclerosis. 2017; 259: 75-82. doi: 10.1016/j.atherosclerosis.
6) Maluf CB, Barreto SM, Giatti L, et al. Association between C reactive protein and all-cause mortality in the ELSA-Brasil cohort. J Epidemiol Community Health. 2020; 74(5): 421-7. doi: 10.1136/jech-2019-213289.
7) Kawamoto R, Kikuchi A, Niomiya D, et al. High-sensitivity C-reactive protein is a predictor of all-cause mortality in a rural Japanese population. J Clin Lab Anal. 2024; 38(4): e25015. doi: 10.1002/jcla.25015.
8) Tabara Y, Setoh K, Kawaguchi T, et al. Association between serum α1-antitrypsin levels and all-cause mortality in the general population: the Nagahama study. Sci Rep. 2021; 11(1): 17241. doi: 10.1038/s41598-021-96833-3.
9) Engström G, Lind P, Hedblad B, et al. Effects of cholesterol and inflammation-sensitive plasma proteins on incidence of myocardial infarction and stroke in men. Circulation. 2002; 105(22): 2632-7. doi: 10.1161/01.cir.0000017327.69909.ff.
10) Engström G, Lind P, Hedblad B, et al. Lung function and cardiovascular risk: relationship with inflammation-sensitive plasma proteins. Circulation. 2002; 106(20): 2555-60. doi: 10.1161/01.cir.0000037220.00065.0d.
11) Engström G, Lind P, Hedblad B, et al. Long-term effects of inflammation-sensitive plasma proteins and systolic blood pressure on incidence of stroke. Stroke. 2002; 33(12): 2744-9. doi: 10.1161/01.str.0000034787.02925.1f.
12) Engström G, Stavenow L, Hedblad B, et al. Inflammation-sensitive plasma proteins, diabetes, and mortality and incidence of myocardial infarction and stroke: a population-based study. Diabetes. 2003; 52(2): 442-7. doi: 10.2337/diabetes.52.2.442.

13) Engström G, Hedblad B, Stavenow L, et al. Fatality of future coronary events is related to inflammation-sensitive plasma proteins: a population-based prospective cohort study. Circulation. 2004; 110(1): 27-31. doi: 10.1161/01.CIR.0000133277.88655.00.

14) de Serres F, Blanco I. Role of alpha-1 antitrypsin in human health and disease. J Intern Med. 2014; 276(4): 311-35. doi: 10.1111/joim.12239.

15) Yamanaka T, Matsumoto S, Teramukai S, et al. The baseline ratio of neutrophils to lymphocytes is associated with patient prognosis in advanced gastric cancer. Oncology. 2007; 73(3-4): 215-20. doi: 10.1159/000127412.

16) Templeton AJ, McNamara MG, Šeruga B, et al. Prognostic role of neutrophil-to-lymphocyte ratio in solid tumors: a systematic review and meta-analysis. J Natl Cancer Inst. 2014; 106(6): dju124. doi: 10.1093/jnci/dju124.

17) Dolan RD, Lim J, McSorley ST, et al. The role of the systemic inflammatory response in predicting outcomes in patients with operable cancer: Systematic review and meta-analysis. Sci Rep. 2017; 7(1): 16717. doi: 10.1038/s41598-017-16955-5.

18) Huang Z, Fu Z, Huang W, et al. Prognostic value of neutrophil-to-lymphocyte ratio in sepsis: A meta-analysis. Am J Emerg Med. 2020; 38(3): 641-7. doi: 10.1016/j.ajem.2019.10.023.

19) Song SY, Zhao XX, Rajah G, et al. Clinical significance of baseline neutrophil-to-lymphocyte ratio in patients with ischemic stroke or hemorrhagic stroke: An updated meta-analysis. Front Neurol. 2019; 10: 1032. doi: 10.3389/fneur.2019.01032.

20) Verdoia M, Schaffer A, Barbieri L, et al. Impact of diabetes on neutrophil-to-lymphocyte ratio and its relationship to coronary artery disease. Diabetes Metab. 2015; 41(4): 304-11. doi: 10.1016/j.diabet.2015.01.001.

21) Templeton AJ, Ace O, McNamara MG, et al. Prognostic role of platelet to lymphocyte ratio in solid tumors: a systematic review and meta-analysis. Cancer Epidemiol Biomarkers Prev. 2014; 23(7): 1204-12. doi: 10.1158/1055-9965.EPI-14-0146.

22) Hu B, Yang XR, Xu Y, et al. Systemic immune-inflammation index predicts prognosis of patients after curative resection for hepatocellular carcinoma. Clin Cancer Res. 2014; 20(23): 6212-22. doi: 10.1158/1078-0432.CCR-14-0442.

23) Porrata LF, Ristow K, Habermann TM, et al. Peripheral blood lymphocyte/monocyte ratio at diagnosis and survival in nodular lymphocyte-predominant Hodgkin lymphoma. Br J Haematol. 2012; 157(3): 321-30. doi: 10.1111/j.1365-2141.2012.09067.x

24) Fest J, Ruiter TR, Groot Koerkamp B, et al. The neutrophil-to-lymphocyte ratio is associated with mortality in the general population: The Rotterdam Study. Eur J Epidemiol. 2019; 34(5): 463-70. doi: 10.1007/s10654-018-0472-y.

25) Song M, Graubard BI, Rabkin CS, et al. Neutrophil-to-lymphocyte ratio and mortality in the United States general population. Sci Rep. 2021; 11(1): 464. doi: 10.1038/s41598-020-79431-7.

26) Mathur K, Kurbanova N, Qayyum R. Platelet-lymphocyte ratio (PLR) and all-cause mortality in general population: insights from national health and nutrition education survey. Platelets. 2019; 30(8): 1036-41. doi: 10.1080/09537104.2019.1571188.

27) Li H, Wu X, Bai Y, et al. Physical activity attenuates the associations of systemic immune-inflammation index with total and cause-specific mortality among middle-aged and older populations. Sci Rep. 2021; 11(1): 12532. doi: 10.1038/s41598-021-91324-x.

28) Hua Y, Sun JY, Lou YX, et al. Monocyte-to-lymphocyte ratio predicts mortality and cardiovascular mortality in the general population. Int J Cardiol. 2023; 379: 118-26. doi: 10.1016/j.ijcard.2023.03.016.

29) Chan SHT, Yu T, Zhang Z, et al. Total and differential white blood cell count and cause-specific mortality in 436 750 Taiwanese adults. Nutr Metab Cardiovasc Dis. 2022; 32(4): 937-47. doi: 10.1016/j.numecd.2021.11.004.

30) Tabara Y, Yamada H, Setoh K, et al. The association between the Moyamoya disease susceptible gene RNF213 variant and incident cardiovascular disease in a general population: the Nagahama study. J Hypertens. 2021; 39(12): 2521-26. doi: 10.1097/HJH.0000000000002964.

31) Janciauskiene S, DeLuca DS, Barrecheguren M, et al. Serum levels of alpha1-antitrypsin and their relationship with COPD in the general spanish population. Arch Bronconeumol. 2020; 56(2): 76-83. doi: 10.1016/j.arbres.2019.03.001.

*Original*

# Current status of quality management of standard 12-leads electrocardiography at ISO 15189 accredited medical institutes in Japan.

*Naoko Ogasawara*[1], *Akiko Tanabe*[6], *Kei Masaki*[2], *Kayoko Asashi*[4], *Tomoyuki Mochida*[2], *Masao Daimon*[5], *Naoyuki Yokoyama*[1], *Naoko Tojo*[3], *Yuichiro Shirota*[2], †*Taiji Furukawa*[1]

†Correspondence: Department of laboratory medicine, Teikyo university school of medicine, 2-11-1, Kaga, Itabashi-ku, Tokyo 173-8605, Japan
Tel: +81-03-3964-1211; Fax: +81-03-3964-7229;
E-mail: tfrkw@med.teikyo-u.ac.jp
Received July 9, 2025; accepted August 23, 2025
*1 Department of laboratory medicine, Teikyo university school of medicine
*2 Department of laboratory medicine, The University of Tokyo Hospital
*3 Clinical laboratory, Sanraku Hospital
*4 Laboratory for clinical investigation, The University of Osaka hospital
*5 Department of Cardiology, Tokyo women's hospital university.
*6 Office of Clinical Laboratory Technology, Keio University Hospital

## ABSTRACT

**Objective:** To investigate the quality management status of standard 12-lead electrocardiography at ISO 15189 accredited institutes in Japan and to support to develop appropriate operational guidelines.
**Methods:** We conducted a questionnaire survey on the status of quality management at each of the institutes certified in the field of electrocardiography, and then conducted an external quality assessment for eight automatic measurement items by distributing waveform simulators and recording control electrocardiograms to those institutes that wished to participate. The target institutes were selected to include various environments to the extent possible (e.g., vendors used, regular maintenance, regions, etc.), and the factors affecting the results were evaluated. In addition, in order to determine the status of internal quality management, all target electrocardiographs were recorded three times a day for at least 10 days at each institute.
**Results:** Thirty institutes were selected, and we were able to conduct the actual survey at 27 institutes between April 2023 and December 2024. The total number of electrocardiographs was 131, and the total number of records was 4052 records. The results revealed that there were inter-instrument and inter-institutional differences in seven automatic measurement items. The vendor used, the filter used during recording, and the presence or absence of vendor maintenance were significant factors influencing the measured values. The X-R control chart for each electrocardiograph showed that when the control limit was set at three times the standard deviation of the measured value, more than one-fourth of the instruments showed fluctuations exceeding this limit for some measurement items.
**Discussion:** Regarding the accuracy control of standard 12-lead electrocardiogram examinations, it has become clear that there are multiple facilities that cannot be evaluated as appropriate in terms of both internal and external accuracy control, and that it is necessary to conduct a more extensive survey and establish appropriate operational guidelines.

〔Lab Med Int 2025; 4(4): 114-124〕

**Key Words**
electrocardiography, quality management, internal quality assessment, external quality assessent, ISO 15189

The electrocardiogram (ECG) is the most widely used physiological test in modern medicine in general. ECG recordings are used to capture the electrical activity of the heart. Abnormalities in this activity can be indicative of heart disease, and the identification of these abnormalities is important for diagnosis and screening. According to the World Health Organization (2024), ischemic heart disease is set to become the leading cause of death in 2021, accounting for 13% of global deaths [1]. Consequently, the early identification of patients at risk, the monitoring of diagnosed patients, and the enhancement of treatment are imperative. ECG recording is regarded as a potent instrument in this regard. Furthermore, contemporary research has investigated innovative approaches for diagnosis and prognostication, emphasizing the application of portable devices and artificial intelligence (AI) in the diagnostic procedure. The quality evaluation of these methods has also been a subject of active research [2) 3)]. However, the majority of research is focused on the evaluation of post-processing methods for obtained or already existing datasets, and there are few reports that mention the quality assurance of acquired signals.

In ECG recording, maintenance and quality management of measurement devices are considered important tasks that directly affect the reported results. However, the current related regulations (Medical Care Law) only state that "routine maintenance and inspection are necessary," but do not provide specific operational guidelines. In addition, with the digital ECG recorder that are currently in widespread use, the scope of institute personnel involvement in maintenance and inspection is limited, and work related to quality management continues to rely on vendors.

On the other hand, in third-party accreditation of clinical laboratories, physiological function tests, including ECG tests, have been included in the scope of ISO 15189 accreditation since 2015, and currently more than 200 institutes have obtained this accreditation. However, according to the results of a survey conducted among accredited institutes since 2020, it cannot be said that the accuracy control activities and accuracy assurance of ECG tests are appropriate. In particular, despite the fact that most institutes use the automatic measurement functions and automatic diagnostic results of ECG recordings as official reports from the department, the complete absence of external quality assessment (EQA) activities was considered a significant issue. Therefore, we planned and implemented the first EQA activity for standard 12-lead ECGs in Japan.

First, we conducted and reported on an EQA alternative approach activity as a feasibility study at six university hospital physiological function testing laboratories, including our own laboratory [4)]. In the report, we found significant inter-institute differences in multiple measurement values. However, because it was a small-scale study, we were unable to obtain significant results regarding the factors affecting the measurement values. In this study, we conducted an EQA activity involving a wider range of participating institutes and examined the above factors. Additionally, since there is little published information on the internal quality acessment status of each institute, we simultaneously conducted a survey over a limited period.

This paper presents the results of an alternative approach to EQA for ECG test at ISO 15189 certified institutes, which was planned by the Physiological Testing Committee of the Japan Association of Clinical Laboratory Science (JCLS) and implemented from 2020 to 2024.

## I. Method

Object: Of the institutes participating in the JCLS Physiology Committee survey in 2023, we contacted 109 institutes that responded "yes" or "yes depending on the content" to the question about participation in external quality control and alternative approach activities, and 79 institutes responded that they could actually participate. Of these, 30 institutes were selected for the survey based on their environment (vendors used, whether or not regular maintenance is required, and whether or not they are located in areas where delivery is possible).

Reference ECG: Three ECG simulators (ESIM-200, Fukuda Denshi, Tokyo) were used for recording. The manufacturer's official accuracy was 0.5% for time intervals rate and 2% for output voltage. The vendor also provided reference values for the measurement items to be measured this time. The simulator was used to record a reference ECG in normal form with a heart rate of 80 beats per minute (bpm).

After calibration by the manufacturer, the simulator was sent to the participating laboratories with instructions for recording. All ECG recorders (electrocardiograph) within the scope of ISO accreditation in each laboratory were evaluated and each recorder was recorded three times a day for at least 10 days. Recording was requested on consecutive days whenever possible, but non-consecutive days were allowed if holidays were included. After recording was completed, the simulators were sent to the next laboratory. Hard copies or measurement data were sent to the laboratory of Teikyo University Hospital, the

administrative office. The recording conditions, including the filter settings of the ECG recorders, were instructed to be the same as the standard 12-lead ECG recordings routinely performed at each institution.

Analysis: In this study, the automated measurements of the reference ECG were used for analysis: heart rate, RR interval, PR interval, QRS width, mean QRS axis, QT interval, $SV_1$, and $RV_5$ amplitudes. The eight measured values were obtained either using values output from the filing system (system output) or reading from the values displayed on the screen or hard copy (visual reading).

Factors affecting the measured values were examined: inter-institute differences, simulator (numbers 1, 2, and 3), intra-day variation, inter-day variation, recorder, data format (system output or visual reading), vendor maintenance, vendors (two companies), and filter settings. Filter settings were classified as on/off for each of the three types of filters (drift, alternating current[AC], and electromyogram) equipped in ordinary ECG recorders, and analyzed in eight levels of classification, from all off to all on. Analysis software was JMP version 18.0 (CA, USA), and univariate and multivariate analyses were used as appropriate for evaluation. Hypothesis testing methods were non-parametric when possible, with a significance level of 5%.

To investigate the internal quality management status, an $\bar{X}$-R control chart was created for each individual ECG recorder, and the occurrence of outliers was evaluated. Since there is no unified guideline for setting control limits, we followed standard quality management procedures and set the upper control limit (UCL) as the value three times larger than the standard deviation from the mean of the measured values obtained by individual ECGs and the lower control limit (LCL) as the value three times smaller than the mean of the measured values obtained by individual ECGs, and the measured values outside this range were considered outliers.

## II. Result

Of the 30 institutes contacted for participation, 3 declined to perform recordings, leaving 27 institutes with evaluable recordings. The vendor of the ECG system was either Vendor F (Fukuda Denshi, 14 institutes) or Vendor N (Nihon-Koden, 13 institutes) at these 27 institutes. The total number of ECG recorders was 131, with a total of 4,052 recordings. Six institutes used no filters at all, seven used only drift, two used only AC, four used both drift and AC, and eight used all drift, AC, and electromyogram filters. System output of measurements was available at 12 facilities, and routine vendor maintenance was performed at 23 facilities.

**Figure 1** shows the actual recordings of the reference ECGs used in this study and the reference values for each measurement. The ECG waveforms are in the normal range with no particular characteristics. Of the eight measurements considered, the heart rate was excluded from the following study because it showed 80 bpm in all the recordings.

**Figure 2** shows the distribution of the seven measurements under consideration. Each measurement does not show a normal distribution, but rather a multimodal distribution. Mean values and 95% prediction intervals are shown in the figure for reference.

**Table 1** shows the results of univariate analysis of the differences between institutes and the factors considered to influence the measurements considered in this study,

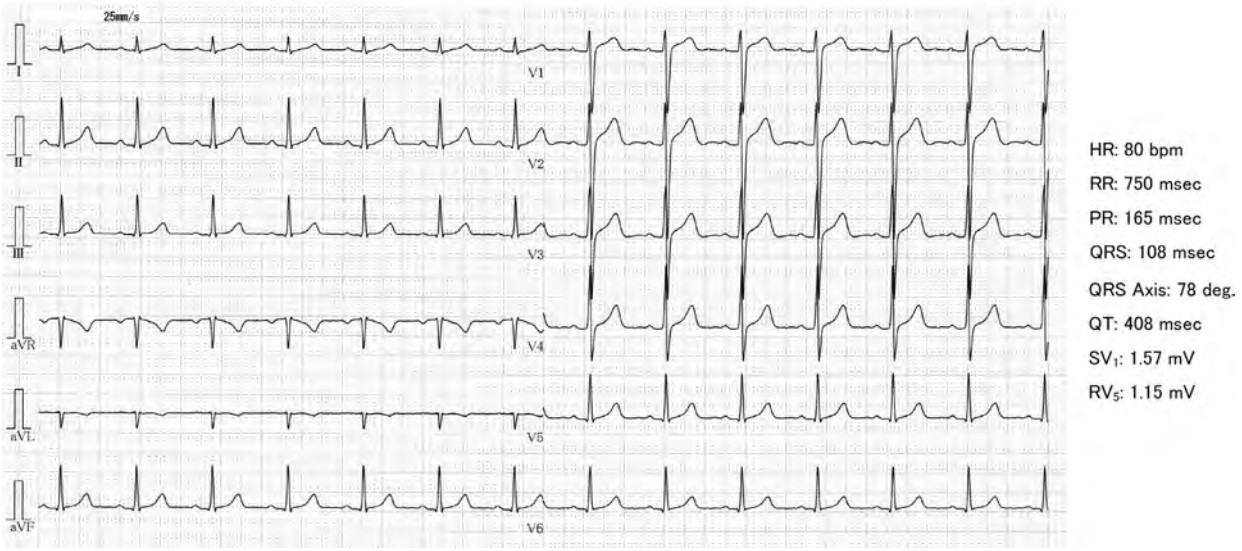


**Figure 1** A recording of control ECG. Reference values of 8 items are presented in right part.

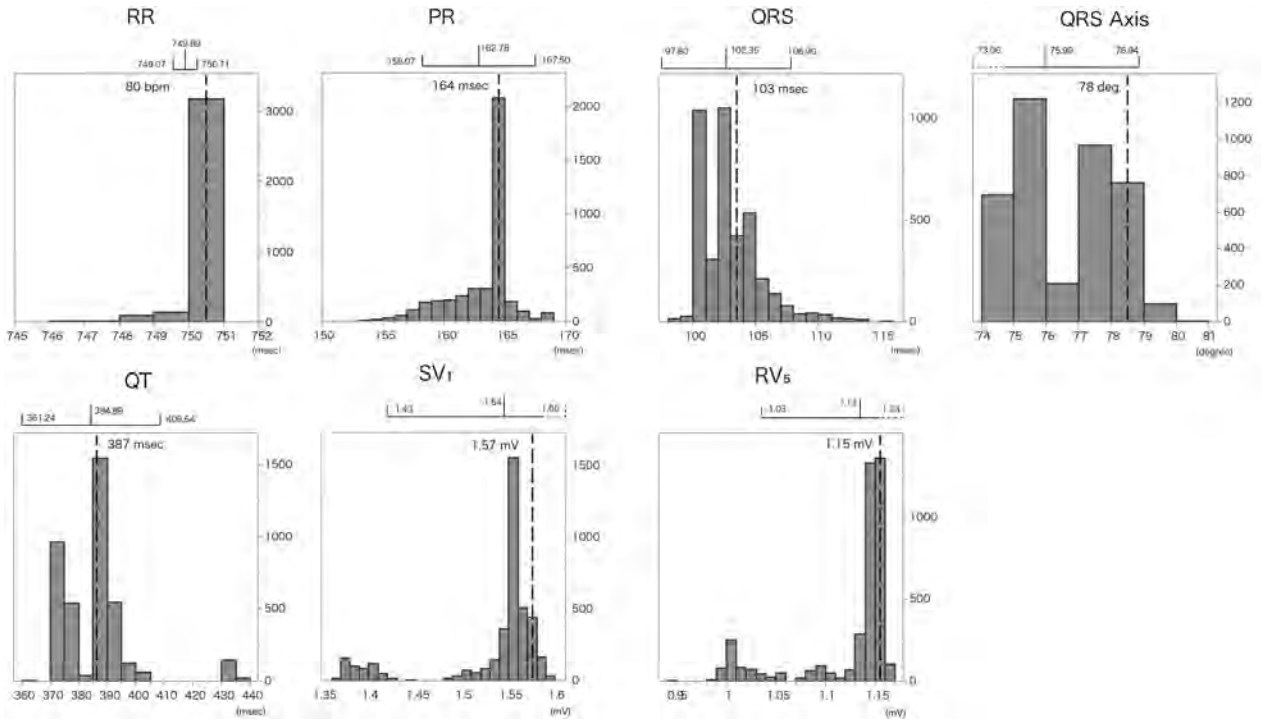


**Figure 2** Histograms of measured values of 7 items in control ECG.
Average values of measured items and 95% prediction intervals as shown in each histogram.

**Table 1** Effects of parameters to measured values.
Nonparametric monovaiate analysis (Kruskal-Wallis test) 21 were performed to each combination.

| | | RR | PR | QRS | Axis | QT | $SV_1$ | $RV_5$ |
|---|---|---|---|---|---|---|---|---|
| Recorder | SSM | 82.052 | 17146 | 12691 | 7609.2 | 576404 | 12.5911 | 9.8386 |
| | *p* | *<.0001* | *<.0001* | *<.0001* | *<.0001* | *<.0001* | *<.0001* | *<.0001* |
| Day to day | SSM | 1.4220 | 16.325 | 30.612 | 7.2006 | 42.610 | 0.0008 | 0.0014 |
| | *p* | 0.5210 | 0.5700 | 0.3401 | 0.0729 | 0.0874 | 0.1774 | *0.0012* |
| Intra-day | SSM | 1.4304 | 0.9750 | 4.8230 | 1.0461 | 1.2100 | 0.0420 | 0.0001 |
| | *p* | 0.1027 | 0.9867 | 0.8332 | 0.6329 | 0.9905 | 0.9990 | 0.9988 |

as well as the results of univariate analysis of each measurement. Significant differences were found in all seven items for inter-institute differences, differences between recorders, and type of filter setting. In addition, all other factors considered had an effect on the measured values. However, no single institute was using multiple vendor instruments and multiple filter settings, and a significant correlation was found between data type/filter setting and Simulator ($p < 0.01$). Therefore, it was not possible to test the independent influence of all factors.

To further examine the factors influencing the measurements in more detail, we next evaluated the internal quality management status of the tests. **Table 2** shows the results of a multivariate analysis using the number of measurements on the same day, date of measurement, and ECG recorder as independent variables. All items showed no significant differences in the number of measurements (intra-day repeatability), but the differences between ECG recorders were significant for all items. As for the variation between measurement days (day-to-day repeatability), significant variation was observed in $RV_5$. Based on these results, $\bar{X}$-R control charts were created for each ECG for each measurement item. **Figure 3** shows an example of $SV_1$. In this figure, both the mean value ($\bar{X}$) and the range of variation (R) for each measurement day show measurements that exceed the control limits (outliers). **Table 3** shows the number of ECGs with measurements exceeding the control limits (the number of ECGs with outliers in $\bar{X}$ or R alone is shown simultaneously), along with the range of individual control limits. The percentage of ECGs with outliers varied by measurement item which ranged from 2-30% of

**Table 2** Results of multivariate analysis for three factors concerning IQA.

| n = 4052 | level | | RR | PR | QRS | Axis | QT | $SV_1$ | $RV_5$ |
|---|---|---|---|---|---|---|---|---|---|
| Institute | 27 | *p* | *<.0001* | *<.0001* | *<.0001* | *<.0001* | *<.0001* | *<.0001* | *<.0001* |
| Recorder | 131 | *p* | *<.0001* | *<.0001* | *<.0001* | *<.0001* | *<.0001* | *<.0001* | *<.0001* |
| Data type | 2 | *p* | *<.0001* | *0.0013* | 0.0856 | *<.0001* | *0.0007* | *<.0001* | 0.2632 |
| Vendor | 2 | *p* | 0.1254 | *<.0001* | *<.0001* | *<.0001* | *<.0001* | *<.0001* | *<.0001* |
| Maintenance | ＋/－ | *p* | 0.1065 | *<.0001* | *<.0001* | *<.0001* | *<.0001* | *<.0001* | *<.0001* |
| Day to day | 10 | *p* | 0.7221 | 0.0220 | *<.0001* | *0.0005* | *<.0001* | *0.0005* | 0.5650 |
| Intra-day | 3 | *p* | *0.0159* | *0.0372* | 0.2764 | *<.0001* | 0.987 | 0.2826 | *<.0001* |
| Filter setting | 5 | *p* | *<.0001* | *<.0001* | *<.0001* | *<.0001* | *<.0001* | *<.0001* | *<.0001* |
| Stimulator | 3 | *p* | *<.0001* | *<.0001* | *<.0001* | 0.0032 | *<.0001* | *<.0001* | *0.0062* |

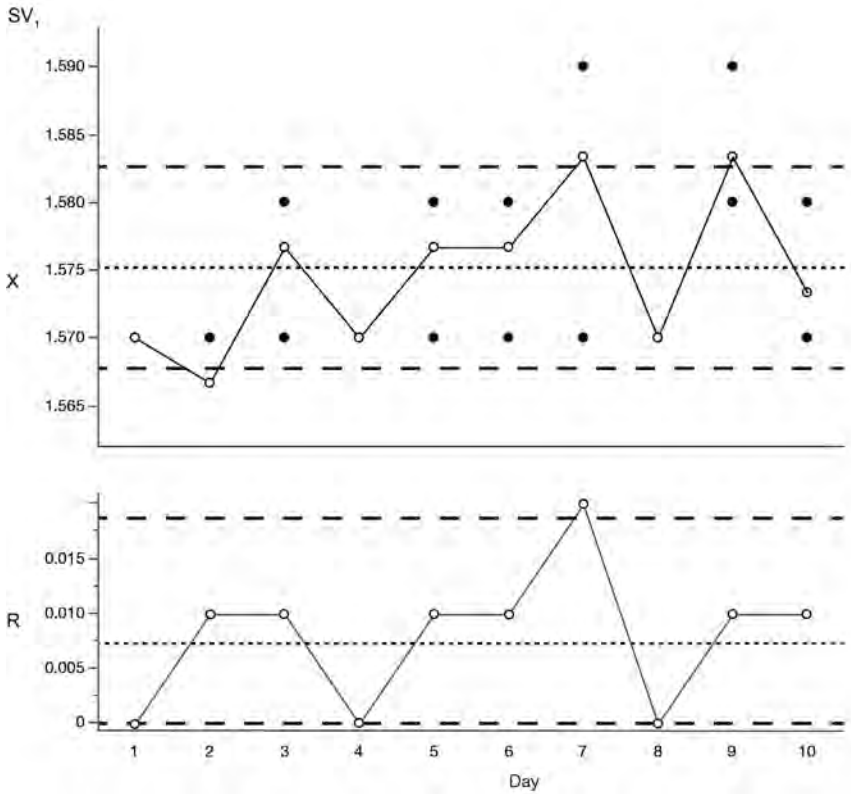


**Figure 3** A sample of $\bar{X}$-R control chart of S wave amplitude in V1 lead. In $\bar{X}$ chart, actual measured values 2 are shown as closed circle and mean values are shown as white circles. Averages of measured values are shown 3 as dotted lines. UCL (upper control limit) and LCL (lower control limit) are shown as dashed lines.

**Table 3** Summary for IQA.
The top of the table shows the number of ECGs in which outliers (total number: either mean or range, and the number of each) appeared. The lower part of the table shows the UCL: upper control limit, LCL: lower control limit, and the minimum and maximum values of the range of variation for each measurement.

| | RR | PR | QRS | Axis | QT | $SV_1$ | $RV_5$ |
|---|---|---|---|---|---|---|---|
| Total | 38 (29.0%) | 34 (26.0%) | 28 (21.4%) | 11 (8.4%) | 15 (11.5%) | 21 (16.0%) | 29 (22.1%) |
| Xbar | 35 (26.7%) | 29 (22.1%) | 21 (16.0%) | 10 (7.6%) | 11 (8.4%) | 19 (14.5%) | 19 (14.5%) |
| R | 36 (27.5%) | 27 (20.6%) | 19 (14.5%) | 3 (2.3%) | 5 (3.8%) | 7 (5.3%) | 17 (13.0%) |
| UCL | 750.1 - 750.6 | 157.4 - 166.2 | 100.5 - 105.2 | 75.1 - 78.4 | 376.0 - 391.7 | 1.42 - 1.59 | 1.00 - 1.17 |
| LCL | 748.3 - 749.9 | 153.6 - 163.9 | 99.1 - 101.5 | 74.2 - 76.9 | 372.3 - 389.0 | 1.40 - 1.57 | 0.98 - 1.16 |
| Range | 0.23 - 2.83 | 0.94 - 6.44 | 1.03 - 6.44 | 0.51 - 2.06 | 1.45 - 11.84 | 0.01 - 0.03 | 0.00 - 0.04 |

the instruments.

Next, we evaluated the inter-laboratory variation of measured values (external quality management survey), which was the original purpose of the study. We examined the factors influencing the differences in detail. As mentioned earlier, it was not possible to examine the independent effects of all factors, so multivariate analysis was performed for the three factors considered important in actual operation: vendor (F or N), regular maintenance (yes or no), and filter settings. **Table 4** shows the results. The three factors had significant effects on all measurement items except the relationship between the presence of regurlar maintenance and the axis.

In particular, the vendor and filter settings were evaluated in all combinations, as they were considered important as findings in actual operations. **Figure 4** shows a plot of each measurement for each of the two ECG filing systems and each filter setting used by the institutes participating in this study. There were five filter settings. For all measurements except RR interval, the dotted line shows the ± 5% reference value of the simulator waveform used in this study. It can be seen that for each measurement value, there are measurements that exceed this value.

## III. Discussion

This survey is the first report on external quality assessment (EQA, proficiency testing) of electrocardiogram (ECG) examinations ever conducted in Japan. Prior to this survey, a feasibility study was conducted at six laboratories as an alternative approach, which indicated the existence of inter-institutional differences in measured values. The results of this study confirm this and provide important insights into the factors that influence measurement values and the status of internal quality assessment (IQA).

Quality management operations of physiological function tests are not yet widespread in general, therefor we investigated the status of IQA of each institute in this survey in addition to EQA. Since it was clear that there were inter-instrumental differences in measurements, as observed in our previous study [4], an $\bar{X}$-R control chart was created for each ECG recorder. As a result, albeit for a limited period and scope, it was found that outliers occurred in nearly 30% of the ECG recorders, depending on the measurement item. This may indicate that daily internal quality management is necessary even for normal ECG recordings. However, since the majority of recorders did not show outliers in many measurement items, it is not necessary to require continuous daily quality management for all recorders and item combinations. It is considered realistic to evaluate the characteristics of each instrument for each item at the time of introduction and to construct internal quality management operations accordingly. In our previous study, for measurements with particularly large daily differences, errors in the lead connection between the ECG recorder and the simulator were presumed from comparisons of recorded waveforms, and such records were excluded from the analysis. Since the original waveforms were not collected in this survey and such operation could not be taken, there is a possibility that leads connection errors were included as a cause of fluctuations in measurements. We believe that it was appropriate to include such recorded values in the analysis for the purpose of the survey. The internal quality control at each institute is required to ensure the accuracy of measurements, including leads connection errors. In addition, it became a very narrow control limit range by the standard method of setting control limits used in this study. When the maximum UCL and minimum LCL values were compared, the RR interval was about 3 msec and the QRS mean electrical axis was about 2 degrees, which should not be a major problem from a clinical perspective. On the other hand, for wave height measurements such as $SV_1$ and $RV_5$, the range is nearly 20% of the wave height for the ECGs with the largest fluctuations, and the case in which daily variations actually affect clinical judgment should be considered. Guidelines for setting reasonable control limits for each measurement item are needed.

The results of the EQA (proficiency testing), which

**Table 4** Results of multivariate analysis for three factors related to the inter-institute differences.

| | | RR | PR | QRS | Axis | QT | $SV_1$ | $RV_5$ |
|---|---|---|---|---|---|---|---|---|
| Vendor | SSM | 15.556 | 5757.0 | 4545.1 | 4346.1 | 56118 | 2.5988 | 2.5241 |
| | *p* | *<.0001* | *<.0001* | *<.0001* | *<.0001* | *<.0001* | *<.0001* | *<.0001* |
| Vendor Maintenance | SSM | 6.4362 | 351.62 | 95.683 | 2.2757 | 20173 | 0.0366 | 0.0903 |
| | *p* | *<.0001* | *<.0001* | *<.0001* | *<.0576* | *<.0001* | *<.0001* | *<.0001* |
| Filter setting | SSM | 15.453 | 5096.5 | 2472.41 | 114.80 | 152965 | 5.5256 | 3.8698 |
| | *p* | *<.0001* | *<.0001* | *<.0001* | *<.0001* | *<.0001* | *<.0001* | *<.0001* |

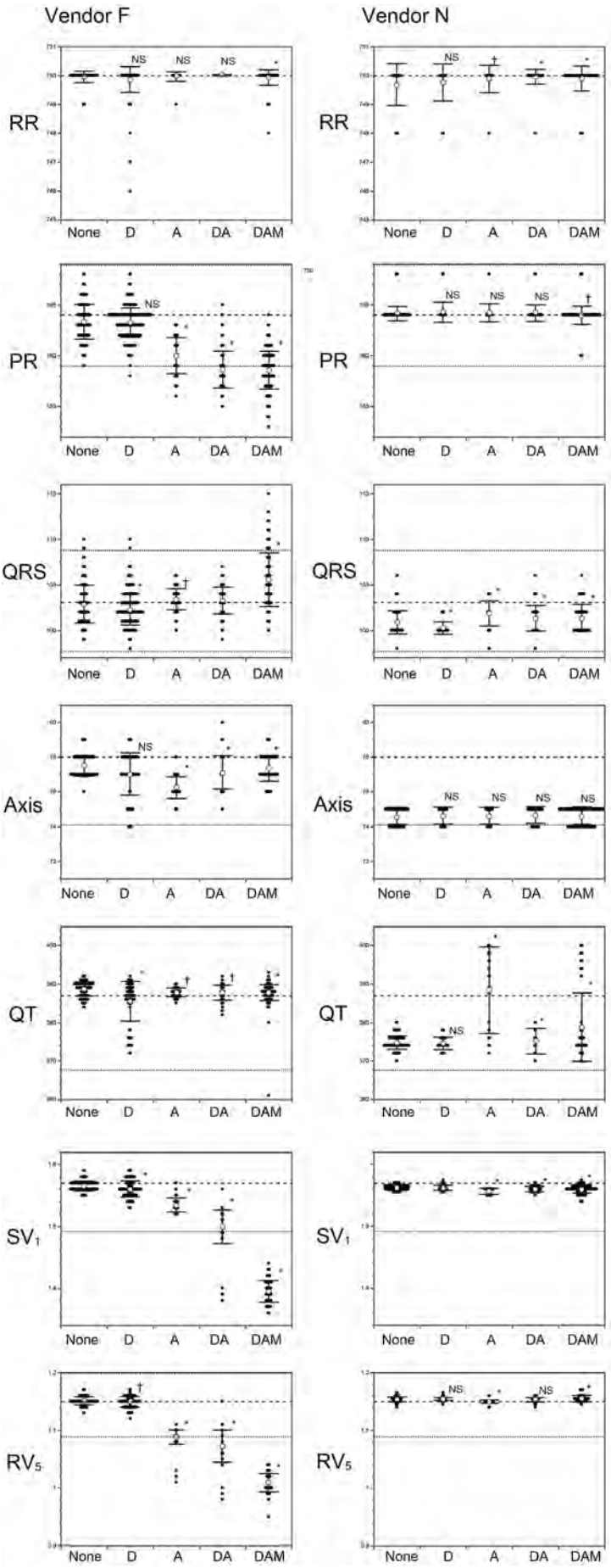


**Figure 4** Distribution of each measurement by filter setting and vendor. The left column shows the recordings from Vendor F and the right column shows the recordings from Vendor N. Each measurement is shown for each of the five filter settings. Actual measured values are shown as dots, mean values are shown as white circles, and standard deviation ranges are shown as solid lines. The simulator's reference value for each measurement item is indicated by a dashed line. If the displayed range exceeds ± 5% of the standard value, this is indicated by a dotted line. The ± 5% range for each measurement is as follows; RR: 712.5-787.5 msec, PR: 155.8-172.2 msec, QRS: 97.85-108.15, Axis: 74.1-81.9, QT: 367.65-406.35 msec, $SV_1$: 1.4915-1.6485 mV, $RV_5$: 1.0925-1.2075 mV. Filter settings are None: none, D: drift filter only, A: AC filter only, DA: drift and AC filters, DAM: all drift, AC and EMG filters. significant difference, the following symbols are appended; *: $p < 0.01$, †: $p < 0.05$.

was the original purpose of this survey, are as follows: 1) The overall automated measurements had variations in both amplitude and time interval that could affect clinical judgment; 2) The variation in measurements was not normally distributed, clearly indicating inter-laboratory differences; and 3) The vendor and filter settings used by each laboratory had a large influence on the measured values.

One of the most important findings of this study is that filter settings have a significant impact on each measurement value on the ECG in actual operating environments. Although it has been technically reported that filter settings have an effect on measured values in digital electrocardiographs that are widely used today [5], users may not fully understand the effect of filter settings. In order to systematically evaluate the degree of influence in the actual operating environment, an external accuracy control evaluation such as the one conducted in this study is necessary, and there are no similar reports except for our previous study. In that study, the number of facilities was too small to analyze the factors affecting this variability. In the present study, however, this analysis was possible by selecting target institutes that included a variety of their environments (filter settings, vendors employed, whether or not regular maintenance was performed, etc.). As shown in **Figure 4**, the filter settings have a very large influence on the error of more than 10% from the reference value, which is a fact that should be understood by all personnel involved in physiological function testing. Of the filter settings examined in this study, the AC and electromyogram filters tended to be discouraged from use because of their widely recognized effects on waveforms. However, the drift filter was used by many facilities (19/27 facilities, 70%). The influence of either filter on the measured values cannot be considered negligible, and caution should be exercised. Even if the use of appropriate filters is unavoidable due to factors such as facility equipment, location, and surrounding environment forces, facilities that include automatic measurements within the scope of ISO 15189 accreditation must make every effort to keep errors in their measurements within acceptable limits (within control limits) as external accuracy control. If a department is unable to keep errors within control limits, it should clearly state that those measurements and automated diagnostic results are outside the scope of ISO 15189 accreditation.

Regarding control limits, the JIS standard for ECG recorder requires that the tolerance be within 5% (both wave height and time) as a standard for instrument calibration using square waves, but does not mention changes in waveforms at the time of reporting or automatic measurement results. However, according to the results of the preceding questionnaire, the measured values and waveforms reported by laboratories are in fact used as they are for clinical judgment. As mentioned in the discussion on internal accuracy control, we believe that a consensus needs to be formed as soon as possible on how to set control limits for each measurement item. Until this is established, it would be appropriate to apply the above JIS standards.

Furthermore, since it is rare for a single institute to use systems from multiple vendors, without conducting a institute-wide evaluation such as this one, it is likely that the difference in the scope of the filter's influence depending on the vendor would not have been clearly recognized. The scope of the filter settings' influence on the recorded data is both waveforms and filing data in the F vendor system, but only waveforms in the N vendor system. Vendor N uses pre-filtered values for filing data. This was confirmed through interviews with each vendor, but we are concerned that neither the personnel who actually use the ECG recorder nor the department members fully understand this.

While it would be desirable from the standpoint of ensuring test accuracy that filter settings have no effect on filing data, this raises another issue. **Figure 5** shows an example of V5 recordings from a institute where all filters are used. The recordings using the recorder of Vendor F on the left side show an R wave height (0.98 mV) that is clearly smaller than the reference value wave height, but the measured value stored in the filing system and the reported actual wave height are equal. On the other hand, the recording using the recorder of Vendor N on the right side shows an R wave height stored in the filing system equal to the reference value (1.15 mV), but the wave height of the actual waveform is clearly smaller (0.99 mV) and comparable to that of Vendor F. Considering that ECG testing is a diagnostic method traditionally based on visual waveform diagnosis, it is difficult to consider a situation where there is a discrepancy between the automatically measured value and the waveform to be desirable. On the other hand, the automatic diagnostic algorithm for ECG recorder first obtains a Minnesota code based on automatically measured wave height and time indices, which is combined with each vendor's additional evaluation indices to improve diagnostic accuracy and sensitivity, leading to the results. Since it is thought that the automatic measurements of each recorder are used to obtain the Minnesota code, it is assumed that the influence of the filter on the filing data will have a large

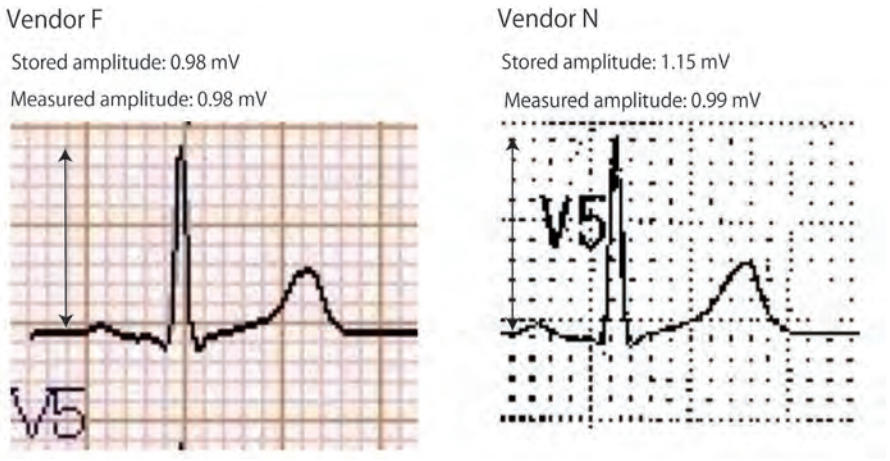


**Figure 5** Sample recordings of control ECG to present discrepancies between wave amplitudes and filed data. See discussion for detail.

impact on the automatic diagnosis results. Although we were not able to evaluate the impact on the automatic diagnosis results this time, the aforementioned survey results [6] indicate that not a few institutes use the automatic diagnosis results for clinical judgment as well, and therefore, it is considered necessary to evaluate the automatic diagnosis results as part of EQA. In addition, as mentioned at the beginning, AI is currently being actively utilized in ECG diagnosis, but unless analysis is conducted after clarifying the effects of filters on the data used, such as waveforms and measurement values, it is unlikely that the expected results will be obtained.

As in our previous study, the limitation of this study is that the survey was conducted only among institutes that had obtained ISO 15189 certification, and the number of institutes was limited to 30, so the information is considered to have selection bias as to the actual status of laboratories in Japan. However, the participating laboratories obtained third-party certification as a department and voluntarily participated in external quality management activities. Therefore, we believe that the results of the survey were obtained from institutes that have a top-level awareness of quality management as physiology laboratories in Japan. The results of the two surveys were almost similar, and the survey method was appropriate. In addition, the results of both the internal and external quality control surveys clearly indicate that there are issues that need to be improved immediately. It is necessary to continue to conduct more extensive and precise quality management surveys.

## Disclosure

No potential conflicts of interest were disclosed.

## Appendics

The institutes contribuled to this work. RML number is an accreditation number distributed by the accreditation body.

| RML number | Institution name |
|---|---|
| RML00480 | Aso Co., Department of Central Clinical Laboratory, Iizuka Hospital. |
| RML00520 | Department of Clinical Laboratories and Transfution Medicine & Cell Therapy and Department of Pathology, Yokohama City University Hospital. |
| RML00620 | Social Walfare Organization Saiseikai Imperial Gift Foundation, Inc. Department of Clinical Laboratory, Osaka Saiseikai Nakatsu Hospital. |
| RML00640 | Division of Blood Transfusion and Cell Therapy, Division of Clinical Pathology, and Department of Clinical Laboratory, Kagoshima University Hospital, Kagoshima University. |
| RML00800 | Hakodate City Hospital Bureau Department of Central Laboratory, Hakodate Municipal Hospital. |
| RML00840 | Keio University, Clinical Laboratory, Center for Transfusion Medicine and Cell Therapy, Diagnostic Pathology, Keio University Hospital. |
| RML00930 | Japanese Foundation for Cancer Researth, Clinical Examination Center/Clinicopathology Center, The Cancer Institute Hospital of JFCR. |
| RML00960 | Osaka University, Laboratory for Clinical Investigation, Department of Blood Transfusion, Department of Diagnostic Pathol- |

ogy, Clinic of Radiology, Ultrasound Diagnostic Center, Osaka University Hospital.

RML00970 Incorporated School of Kurume University, Department of Clinical Laboratory Medicine, Department of Pathology, Kurume University Hospital.

RML01020 Teikyo University, Department of Clinical Laboratory, Teikyo University Hospital.

RML01030 Hyogo Medical University Hospital, Department of Clinical Laboratory, Center for Transfusion Medicine and Cellular Therapy, Ultrasound Imaging Center, Department of Surgical Pathology, Department of Clinical Technology.

RML01040 Nagoya University, Department of Clinical Laboratory, Department of Blood Transfusion Service, Department of Pathology, Nagoya University Hospital.

RML01070 National University Corporation Gunma University, Department of Clinical Laboratory, Gunma University Hospital.

RML01080 Chiba University, Department of Laboratory Medicine, Department of Pathology, Department of Transfusion Medicine and Cell Therapy, Chiba University Hospital.

RML01100 National University Corporation, University of Miyazaki, Department of Clinical Laboratory and Division of Pathology, University of Miyazaki Hospital.

RML01200 Department of Clinical Laboratory, Kyorin University Hopital, Kyorin Foundation.

RML01240 Japanese Red Cross Society, Japanese Red Cross Ashikaga Hospital Clinical Laboratory Department.

RML01280 Central Clinical Laboratory, Blood Transfusion Section, Pathology Section, General Diagnostic Imaging Center, Diagnostic Pathology Nara Medical Univesity Hospital.

RML01450 Aichi Medical University Corporation, Department of Clinical Laboratory, Department of Transfusion Mdicine, Department of Infection Control and Prevension, Department of Pathological Center, Aichi Medical University Hospital.

RML01500 Medical Laboratory, National Hospital Organization Saitama National Hospital.

RML01570 Gakkouhoujin Dokkyo Gakuen, Dokkyo Medical University Saitama Medical Center, Clinical Laboratory and Transfusion Service and Pathology.

RML01680 Department of Laboratory Medicine, Transfusion and Pathology, Saga University Hospital.

RML01770 Department of Clinical Laboratory, Tohoku Madical and Phamaceutical University Hospital, Tohoku Medical and Pharmaceutical University.

RML02270 Local Independent Administrative Agency Gifu Prefectural General Medical Center, Department of Central Clinical Laboratory, Department of Pathology.

RML02360 Japanese Red Cross Society, Japanese Red Cross Kumamoto Hospital, Department of Clinical Laboratory, Department of Pathology.

RML02480 Japan community health organization, Tokuyama chuo hospital ,clinical laboratory department.

RML02500 Iwate Medical University, Division of Central Clinical Laboratory and Division of Diagnostic Pathology,Iwate Medical Universiy Hospital.

RML02800 Social Walfare Organization Saiseikai Imperial Gift Foundation, Inc. Department of Clinical Laboratory,Saiseikai Kumamoto Hospital.

RML02850 University of Occupational and Environmental Health Department of Laboratory and Transfusion Medicine, Department of Pathology and Laboratory Medicine, Department of Surgical Paholoty, Hospital of University of Occupational and Environmental Health.

RML03100 Division of Central Laboratory, Division of Clinical Pathology, Dividion of Transfusion Medicine, Akita University Hospital, Akita University.

**References**

1) World Health Organization. The top 10 causes of death. <https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death>

2) Qayyum SN, Iftikhar M, Rehan M, et al. Revolutionizing electrocardiography: the role of artificial intelligence in modern cardiac diagnostics. Ann Med Surg (Lond). 2025; 87(1): 161-70.

3) Van der Bijl K, Elgendi M, Menon C. Automatic ECG quality assessment techniques: A systematic review. Diagnostics 2022; 12(11): 2578.

4) Ohmura N, Tomihara T, Sato H, et al. Accuracy of standard

12 lead electrocardiograph recorders used in 6 university hospitals in Japan evaluated by inter-laboratory comparison survey. Rinsho Byori 2019; 67(11): 1109-18.

5) Bailey JJ, Berson AS, Garson A, Jr., et al. Recommendations for standardization and specifications in automated electrocardiography: bandwidth and digital signal processing. A report for health professionals by an ad hoc writing group of the Committee on Electrocardiography and Cardiac Electrophysiology of the Council on Clinical Cardiology, American Heart Association. Circulation 1990; 81(2): 730-9.

6) Ogasawara N, Okano T, Omoto K, et al. The current status of quality management of physiological tests in ISO 15189 accreditated institutes in Japan. Clinical Laboratory Science Journal 2022; 47(5): 598-615.

*Short Communication*

# Effects of Assigning a Risk Manager in the Clinical Laboratory on the Management of Critical Values at Tohoku Medical and Pharmaceutical University Hospital

*Ririka Miura*[*1], Rikei Kozakai*[*1], Yasuhiro Endo, MD, PhD*[*1,2],*

*†Shinichiro Takahashi, MD, PhD *[*1,2]*

†Correspondence: Division of Laboratory Medicine, Faculty of Medicine, Tohoku Medical and Pharmaceutical University, 1-15-1 Fukumuro, Miyagino-ku, Sendai 983-8536, Japan
Tel: +81-22-290-8889; Fax: +81-22-290-8889;
E-mail: shintakahashi@tohoku-mpu.ac.jp
Received May 20, 2025; accepted August 19, 2025
*1 Department of Clinical Laboratory, Tohoku Medical and Pharmaceutical University Hospital, 1-12-1 Fukumuro, Miyagino-ku, Sendai 983-8512, Japan
*2 Division of Laboratory Medicine, Faculty of Medicine, Tohoku Medical and Pharmaceutical University, 1-15-1 Fukumuro, Miyagino-ku, Sendai 983-8536, Japan

**ABSTRACT**

**Objective:** Effective management of medical treatment information, particularly critical values, is essential in every clinical laboratory. A proposal for the management of critical values was issued by the Japanese Society of Laboratory Medicine in 2021. Our laboratory began implementing the revised proposal in December 2024, which recommends appointing a medical technologist as a risk manager. The risk manager is responsible for monitoring the occurrence of critical values, and acting as a liaison between the Clinical Laboratory and the Medical Safety Department.

**Methods:** We evaluated the impact of sharing and reviewing critical values, by a risk manager, with the Medical Safety Department by analyzing the number and content of critical value reports and tracking telephone communications from laboratory physician to attending physicians. Data from the fiscal year 2018 through June 2025 were included in the analysis.

**Results:** In our hospital, the total number of tests increased from 2,301,813 in 2018 to 3,235,049 in 2024. Between April 2018 and November 2024, there were 10 telephone calls from laboratory physician to attending physicians regarding 28,980 critical values. In contrast, from December 2024 onward, there were 13 such calls for just 2,495 critical values—a statistically significant increase ($z = -8.65$, $p < 0.0001$) following the appointment of a risk manager.

**Conclusions:** The introduction of a risk manager to facilitate the sharing and review of critical values with the Medical Safety Department led to a significant increase in follow-up telephone communication from laboratory physician. This may reflect improved professional oversight and laboratory management.

〔Lab Med Int 2025; 4(4): 125-131〕

**Key Words**
critical value, risk management, medical safety

## I. Introduction

In clinical laboratories, the frequency of errors is considered to be lower than in other hospital departments. However, even a low error rate can still result in a considerable number of incidents due to the large volume of tests performed daily [1]. Therefore, ensuring medical safety is essential not only in high-risk areas but also

in all departments, including clinical laboratories. The 2007 medical service law required medical institutions to prepare guidelines for various aspects of hospital operation, including maintenance plans for medical equipment, procedures for pharmaceutical use, infection control, and importantly, medical safety [2]. As a result, medical safety guidelines are now seen as necessary tools to support the delivery of accurate and reliable clinical laboratory data to physicians, and efforts to enhance medical safety have attracted growing attention [3][4].

Our laboratory, the Department of Clinical Laboratory at Tohoku Medical and Pharmaceutical Hospital, obtained ISO 15189 accreditation in January 2019 [5]. Thereafter, we experienced, and reported in this journal, that the implementation of ISO 15189 led to a significant improvement in risk management, contributing to enhanced medical safety and a reduction in the number of incidents [6][7].

In 2021, the Japanese Society of Laboratory Medicine issued a proposal for managing critical values, which was revised in 2024 [8]. Our laboratory, started following the proposal for critical values from December 2024, which recommends to appoint a medical technologist as a risk manager to monitor the number of critical values occurred, review and act as a conduit between Clinical Laboratory, and Medical Safety Department. We report here, the significant increment of checking telephone call from laboratory physician, which may reflect more professional laboratory management.

## II. Methods

### A. Participants and statistics

We analyzed the number of critical values that occurred in the Department of Clinical Laboratory at Tohoku Medical and Pharmaceutical University Hospital. The definition of critical values used in this hospital followed the criteria described previously [9]. The number of critical values observed during the study period was 28,980 from April 1, 2018 to November 30, 2024, and 2,495 from December 2024 to June 30, 2025. For statistical analysis, a z-test was performed to evaluate the significance of differences, and statistical significance was determined accordingly.

### B. Ethics

We carefully considered the protection of personal information. This study focused exclusively on the number of tests and critical values, and did not involve any patient-identifiable data. We strictly adhered to the Policy on the Handling of Case Reports issued by the Ethics Committee of Tohoku Medical and Pharmaceutical University Hospital.

## III. Results

### *1. Transition of the number of the critical values and critical values commenting reports*

Our hospital has undergone several transitions over the years. In 2013, it changed its name from Tohoku Welfare Pension Hospital to Tohoku Pharmaceutical University Hospital (466 beds). Following the establishment of the medical faculty in 2016, the hospital was renamed Tohoku

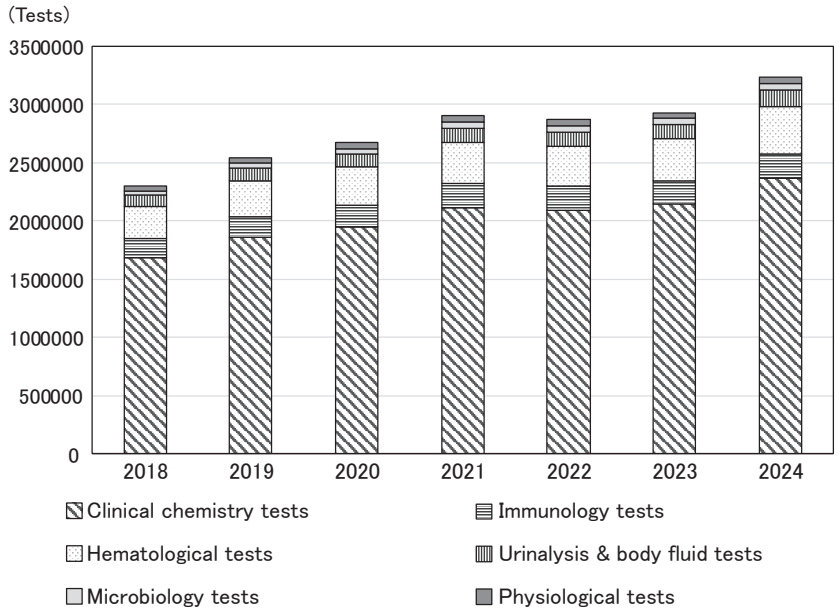


**Figure 1** Annual changes of the transition of the total number of tests

Medical and Pharmaceutical University Hospital. The number of beds increased to 600 in 2020. In this way, the hospital has gradually evolved from a community hospital to a university hospital, with a unique background and changing roles and functions. Annual changes in the total number of tests (including both specimen and physiological tests) during the study period are shown in **Figure 1**. The actual numbers were as follows (by fiscal year): 2018: 2,301,813, 2019: 2,545,856, 2020: 2,670,728, 2021: 2,906,752, 2022: 2,873,229, 2023: 2,931,994, 2024: 3,235,049. Overall, the number of tests increased approximately 1.4-fold during this period.

The management of critical values in our laboratory is carried out as follows. Critical values [9] are reported through the electronic medical record system and by telephone calls from medical technologists to the physicians who ordered the test. All reports are documented in forms called "phone call record memos" (S **Figure 1**). The laboratory physician checks these memos several times a day and confirms, through the electronic medical records, whether the attending physician has taken any action regarding the critical value. If no action is observed in response to a critical value involving antibiotic-resistant bacteria, the laboratory physician issues a "critical values commenting report." For other types of critical values, if no action is taken, the laboratory physician directly calls the attending physician and issues a "critical values commenting report" if necessary (S **Figure 2**).

Until June 2020, the number of critical value items was extremely high at 64 (national average: 19.9) [9], and approximately 7,000 reports of critical values were made annually via telephone (national average: 2,472) [9]. Therefore, a review of the critical value reporting criteria was conducted to ensure appropriate operation. In June 2020, the definition of critical values was revised, reducing the number of items by 18 [10], which clearly led to a decrease in daily reported critical values.

Annual changes in the number of critical values, categorized by medical service, are shown in **Figure 2**. The number of annual critical values clearly decreased after 2020. After June 2020, most of the "critical values commenting reports" were related to antibiotic-resistant bacteria, and their numbers gradually declined (S **Figure 3**). This may indicate that documentation of pathogens in electronic medical records by the attending physicians has become standardized, thereby reducing the need for laboratory physician to issue these reports frequently.

As shown in **Figure 3**, before June 2020, roughly 30 to 40 critical values were reported daily. After the revision in June 2020, the number decreased to about 10 to 20 per day. During the period from April 2018 to November 2024, the number of checking telephone calls made by laboratory physician was 10 (out of 28,980 critical values), whereas after December 2024, when appointed a medical technologist as a risk manager to monitor the number of critical values, the number increased to 13 (out of 2,495). Notably, the proportion of such calls increased significantly, with statistical significance ($z = -8.65$, $p < 0.0001$).

### *2. Transition of the checking telephone call from laboratory physician*

As noted in the Introduction, in December 2024, we appointed a medical technologist as a risk manager to monitor the number of critical values, conduct reviews,

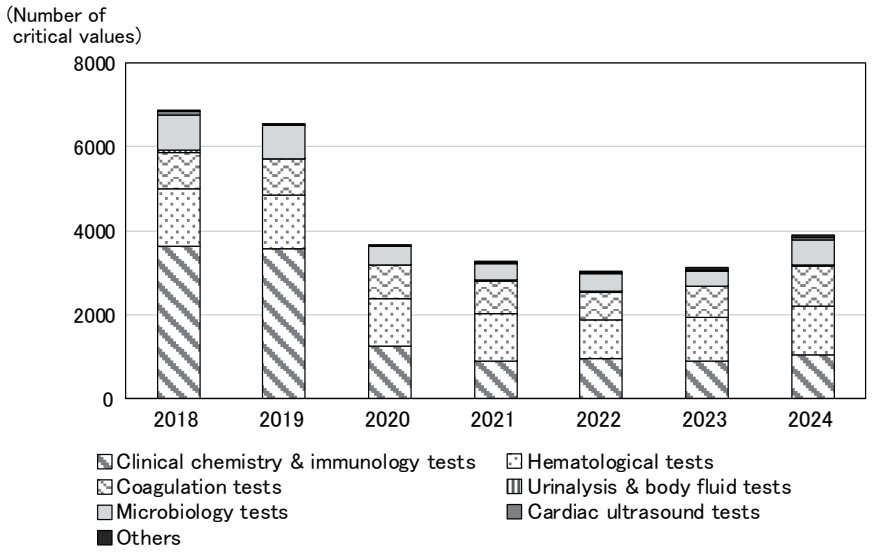


**Figure 2** Transition of the number of the critical values.

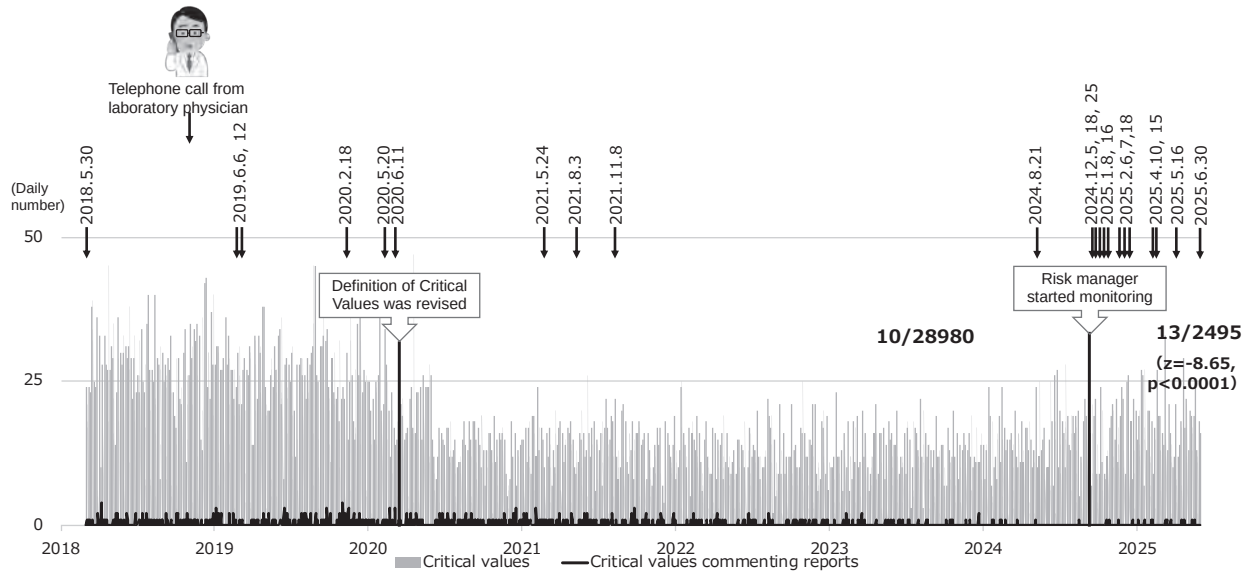


**Figure 3** Transition of the number of the critical values, critical values commenting reports and checking telephone call from laboratory physician.

and act as a conduit between the Clinical Laboratory and the Medical Safety Department. The details of a phone call from 2018 are described in **Table 1** (S **Figure 4**). Among the confirmation calls made by laboratory physician during this period, those concerning potassium concentration (n=8) were the most frequent. Other calls were related to white blood cell morphology and/or counts (n=4), platelet number (n=3), possible M-protein (n=2), flow cytometry results (n=2), coagulation and fibrinolysis (n=2), blood glucose levels (n=1), and antibiotic-resistant bacteria (n=1). Among these calls, on February 18, 2020, we encountered a case in which the laboratory physician contacted the attending physician, who was unaware of a critically low platelet count (Plt = 6,000/μL). This occurred because, laboratory technologists typically notify the physician who ordered the test. However, in this instance, the attending physician differed from the ordering physician, and the notification failed to reach the appropriate recipient. In May 2025, serum protein electrophoresis revealed a possible M-protein in a patient managed by a resident physician in a certain department. Although the resident had been informed of the result, they were uncertain about the appropriate next steps, and no follow-up action was taken. The following day, the laboratory physician contacted the resident, advising additional diagnostic orders and referral to hematology, which subsequently led to a diagnosis of multiple myeloma. A similar incident occurred again in June. These cases suggest that a certain proportion of phone calls made by laboratory physicians may indeed contribute to patient safety. Therefore, the confirmation calls made by laboratory physician serve as a final safeguard.

## IV. Discussion

The definition of critical values has been debated by laboratory professionals for decades [11]. Lippi et al. summarized recommendations regarding the notification, parameters, and communication of critical laboratory values based on available guidelines from the Joint Commission, Clinical and Laboratory Standards Institute, and the Royal College of Pathologists [12]. Although there is some agreement on the definition of critical values, they remain relatively heterogeneous worldwide [12], and no universally accepted definition or list of parameters exists. They are typically defined locally in agreement with relevant stakeholders.

In this context, Doering et al. examined five years of inpatient admissions for critical or near-critical results. They found that elevated potassium thresholds effectively identified patients at risk of death. Furthermore, they clarified that the mortality risk associated with most critical values is time-dependent [13]. Murakami et al. investigated the importance of various clinical test items (glucose, BUN, sodium, potassium, AST, ALT, LDH, CK, phosphate, calcium, serum amylase, white blood cell count, hemoglobin, platelets, PT-INR) by conducting a questionnaire survey among physicians. They found that abnormal results for glucose, PT-INR, and potassium concentration were most frequently regarded as "critical values requiring immediate medical intervention" [14]. In our study, among the checking calls made by laboratory

**Table 1** Details of telephone call from laboratory physician

| Date | Details of phone calls from laboratory physician | At the time laboratory physician called, the physician in charge already noticed the critical value(s)? |
|---|---|---|
| 30-May-18 | Low platelet count, Plt=23000(/μL) (Due to rapid decrease, adding coagulation tests were recommended for further evaluation). | Yes |
| 21-Dec-18 | Provision of advisory services (about reticulated platelets). | N/A |
| 6-Jun-19 | High concentration of potassium, 6.4 (mEq/L), and confirmed the response of the doctor. | Yes |
| 12-Jun-19 | Emergency report about the interpretation of READ flow cytometry result | Yes |
| 18-Feb-20 | Low platelet count, Plt=6000(/μL) and confirmed the response of the doctor. | No |
| 20-May-20 | Emergency report about the results of outsourced flow cytometry analysis, which suggests bone marrow infiltration of mantle cell lymphoma | Yes |
| 11-Jun-20 | Microbiology results after discharge, about MRSA(2+:sputum)、E.coli ESBL(4+:urine from a catheter) | Yes |
| 17-Feb-21 | Provision of advisory service (about white blood cell trends before and after dialysis) | N/A |
| 24-May-21 | Low platelet count, Plt=5000(/μL) and confirmed the response of the doctor. | Yes |
| 15-Jul-21 | Provision of advisory service (about bone marrow examination report). | N/A |
| 26-Jun-21 | Provision of advisory service (about required amount of blood for IgE measurement test). | N/A |
| 3-Aug-21 | Addition of test items [FCM, sIL2R] based on abnormalities in peripheral blood smear | Yes |
| 6-Aug-21 | Provision of advisory service (about interpretation of the result of flow cytometry issued on Aug 3) | N/A |
| 8-Nov-21 | Explain the contents of the peripheral blood smear report. Inform that chronic lymphocytic leukemia (CLL) is highly suspected. | Yes |
| 15-Mar-22 | Provision of advisory service (about pseudohyperkalemia and offered advice on differential diagnosis). | N/A |
| 28-Apr-22 | Provision of advisory service (about suspected macro-CKemia). | N/A |
| 17-May-22 | Provision of advisory service (about macro-CKemia). | N/A |
| 21-Aug-24 | Notified that degranulated neutrophils were observed and advised consideration of hematologic neoplasms with dysplasia, such as CMML. | Yes |
| 18-Nov-24 | Provision of advisory service (about peripheral blood lymphocyte morphology). | N/A |
| 5-Dec-24 | High concentration of potassium, 6.1 (mEq/L), and confirmed the response of the doctor. | Yes |
| 18-Dec-24 | High concentration of potassium, 6.2 (mEq/L), and confirmed the response of the doctor. | Yes |
| 25-Dec-24 | Low concentration of potassium, 2.6 (mEq/L), and confirmed the response of the doctor. | Yes |
| 8-Jan-25 | High concentration of potassium, 6.3 (mEq/L), and confirmed the response of the doctor. | Yes |
| 16-Jan-25 | High concentration of D-dimer 12.92 (μg/mL), and confirmed the response of the doctor. | Yes |
| 6-Feb-25 | High concentration of D-dimer 11.75 (μg/mL), and confirmed the response of the doctor. | Yes |
| 7-Feb-25 | Low concentration of potassium, 2.5 (mEq/L), and confirmed the response of the doctor. | Yes |
| 7-Feb-25 | Low concentration of potassium, 2.5 (mEq/L), and confirmed the response of the substitute doctor. | Yes |
| 18-Feb-25 | High blood glucose of 355 mg/dL and potassium level of 6.3 mmol/L and confirmed the response of the doctor. | Yes |
| 10-Apr-25 | Increased number of WBC, 24000/μL, and confirmed the response of the doctor. | Yes |
| 15-Apr-25 | Severe mitral regurgitation diagnosed by cardiac ultrasound test | Yes |
| 16-May-25 | A possible M-protein was reported. With no response from the resident, immunotyping, IgG, IgA, IgM testing, and hematology referral were recommended. | Yes (but needs guidance) |
| 30-Jun-25 | A possible M-protein was reported. Only urine protein test was ordered from the resident, immunotyping, IgG, IgA, IgM testing, and hematology referral were recommended. | Yes (but needs guidance) |

physician, those related to potassium concentration were the most frequent (**Table 1**). Therefore, it may be necessary to handle results for potassium, glucose, and PT-INR with particular care, ensuring that prompt action is taken by the attending physicians. Further attention and improvement in the communication of these critical values are essential for enhancing medical safety.

It should be noted that various factors can affect serum potassium levels, including elevated white blood cell or platelet counts and pre-analytical factors such as hemolysis or delayed centrifugation during specimen processing [15]. Therefore, laboratory staff should always consider these factors and may need to provide guidance on interpreting such results.

Several studies have shown that multidisciplinary collaboration is necessary to ensure that all critical values are responded to appropriately. One report indicated that in the intensive care unit (ICU), implementation of a daily goals form—which enhanced communication among nurses and residents—reduced ICU length of stay from a mean of 2.2 days to 1.1 day [16]. Fazzini et al. also emphasized the value of regular multidisciplinary safety briefings, which improved situational awareness and nearly halved delays in critical care referrals [17].

In conclusion, sharing and reviewing critical values with the Medical Safety Department through a designated risk manager led to a significant increase in checking telephone calls from laboratory physician. This may reflect a more professional approach to laboratory management.

**Funding**

This work was supported in part by the Shino-Test Corporation and the Shionogi Corporation Research Fund.

**Author contributions**

R. Miura, played a pivotal role for this study, as a risk manager to monitor the critical value, acting as a liaison between our laboratory and medical safety department. R. Miura also analyzed the data and designed the figures. R. Kozakai contributed to manage critical value reporting system. Y. Endo analyzed the data. S. Takahashi designed the study and wrote the manuscript. All authors have accepted responsibility for the entire content of this manuscript and have approved its submission.

**Disclosure of Conflicts of Interest**

None

**References**

1) Kalra J. Medical errors: impact on clinical laboratories and other critical areas. Clin Biochem. 2004; 37(12): 1052-62.
2) Outline of revised health care low. Enforced on April, 2007. Downloaded from 〈https://www.mhlw.go.jp/shingi/2007/11/dl/s1105-2b.pdf〉 (accessed on Dec30, 2024)
3) Tsunekawa K, Asai Y, Ikegami S, et al. Incident analysis and preventive measures taken in the general laboratory of hospital. Jap J Med Tech. 2019; 68(2): 333-38.
4) Maeda F, Izumi S, Nakanishi H, et al. Efforts of inspection technology department and medical safety management committee: Incident accident report, positive report, fiscal year safety target. Jap J Med Tech. 2017; 66(4): 404-10.
5) Ohba Y, Kozakai R, Izumi Y et al. Efforts to Gain Accreditation for ISO 15189 in the Department of Clinical Laboratory, Tohoku Medical and Pharmaceutical University Hospital. Rinsho Byori. 2018; 66(12): 1341-47.
6) Miura R, Kozakai R, Suzuki S, et al., Analysis of Incident/Accident Reports in the Department of Clinical Laboratory at Tohoku Medical and Pharmaceutical University Hospital: Effect of ISO 15189 Implementation on Medical Safety. Lab Med Int. 2024; 3(1): 8-14.
7) Miura R, Kozakai R, Takahashi S. The effect of ISO 15189 implementation for the reduction of incidents. Lab Med Int. 2024; 3(2): 26-7.
8) Japanese Society of Laboratory Medicine. Proposal for the management of critical values. Downloaded from 〈https://www.jslm.org/committees/team_med/20240610-1.pdf〉 (accessed on Jan 1, 2025).
9) Japanese Society of Laboratory Medicine. National critical value survey 2017. Downloaded from 〈https://www.jslm.org/committees/team_med/panic_2017.pdf〉 (accessed on Apr 24, 2025)
10) Kozakai R, Takahashi S. How can we deal with critical values? From the laboratory in major hospitals (Japanese), Med Technol, 2024; 52(1): 22-9.
11) Piva E, Plebani M. From "panic" to "critical" values: which path toward harmonization? Clin Chem Lab Med. 2013 Nov; 51(11): 2069-71.
12) Lippi G, Mattiuzzi C. Critical laboratory values communication: summary recommendations from available guidelines, Ann Transl Med. 2016; 4(20): 400.
13) Doering TA, Plapp F, Crawford JM. Establishing evidence base for critical laboratory value thresholds. Am J Clin Pathol. 2014; 142(5): 617-28.
14) Murakami Y, Nishikawa M. Current Status and Challenges of Panic Value Reporting. J Jap Soc Lab Med. 2021; 69(12): 937-40.
15) Meng QH, Wagar EA. Pseudohyperkalemia: a new twist on an old phenomenon. Crit Rev Clin Lab Sci. 2015; 52(2): 45-55.
16) Pronovost P, Berenholtz S, Dorman T, et al. Improving

communication in the ICU using daily goals. J Crit Care. 2003; 18(2): 71-5.
17) Fazzini B, McGinley A, Stewart C. A multidisciplinary safety briefing for acutely ill and deteriorating patients: A quality improvement project. Intensive Crit Care Nurs. 2023; 74: 103331.

*Short Communication*

# Genetic Variability in SARS-CoV-2 Vaccine Response: Association of rs3824949 in *TRIM5* With Antibody Titers in a Japanese Cohort

*§Satomi Nagaya*[1], *§Nguyen Hoang Thao Giang*[1], *J. Luis Espinoza*[2], *Koichi Yamaguchi*[1,3], *Kota Goto*[4], *Hisanori Horiuchi*[4], *†Eriko Morishita*[1,5]

†Correspondence: Department of Clinical Laboratory Science, Division of Health Sciences, Graduate School of Medical Science, Kanazawa University, 5-11-80 Kodatsuno, Kanazawa, Ishikawa 920-0942, Japan
Tel: +81-76-265-2606; Fax: +81-76-265-2606;
E-mail: eriko86@staff.kanazawa-u.ac.jp
Received July 4, 2025; accepted September 16, 2025

§These authors contributed equally to this work
*1 Department of Clinical Laboratory Science, Division of Health Sciences, Graduate School of Medical Science, Kanazawa University, 5-11-80 Kodatsuno, Kanazawa, Ishikawa 920-0942, Japan
*2 Faculty of Health Sciences, Kanazawa University, 5-11-80 Kodatsuno, Kanazawa, Ishikawa 920-0942, Japan
*3 Department of Medical Technology, Faculty of Health Sciences, Tsukuba International University, 6-20 Manabe, Tsuchiura, Ibaraki 300-0051, Japan
*4 Department of Molecular and Cellular Biology, Institute of Development, Aging and Cancer, Tohoku University, 4-1 Seiryo-machi, Aoba-ku, Sendai, Miyagi 980-8575, Japan
*5 Department of Hematology, Kanazawa University Hospital, 13-1 Takara-machi, Kanazawa, Ishikawa 920-8640, Japan

## ABSTRACT

Individual immune responses to SARS-CoV-2 vaccination vary considerably; however, the genetic factors influencing antibody production remain incompletely understood. This study investigated the association between genetic polymorphisms and anti-SARS-CoV-2 antibody levels in 405 healthy Japanese individuals three weeks after their first dose of an mRNA vaccine. We focused on three genetic polymorphisms previously associated with antibody responses in humans. The GG genotype of rs3824949 in *TRIM5* was significantly associated with higher antibody titers ($P = 0.0009$), whereas rs4792800 in *TNFRSF13B* and rs1611350 in *HLA-F-AS1* showed no significant associations. Stepwise regression analysis confirmed that rs3824949 remained an independent predictor after adjusting for age, lymphocyte count, and platelet count. Given the role of *TRIM5* in innate immunity, our findings suggest that rs3824949 may enhance immune responses to SARS-CoV-2 mRNA vaccination, highlighting the role of host genetics in individual variability in vaccine responses. By identifying a genetic factor that significantly influences antibody responses, this study lays the groundwork for personalized vaccine strategies tailored to individual genetic backgrounds. Moreover, these findings may have broader implications beyond SARS-CoV-2, informing the design and optimization of future mRNA vaccines targeting other infectious diseases or cancers. Our work highlights the potential of precision vaccinology and emphasizes the need to include ethnically diverse populations in immunogenetic research. These insights contribute to a more equitable and effective global vaccination strategy, particularly in the era of rapidly advancing mRNA vaccine technologies.

〔Lab Med Int 2025; 4(4): 132-140〕

**Key Words**
COVID-19, gene association studies, immune response; SARS-CoV-2 vaccine, tripartite motif-containing 5α (TRIM5α)

## I. Introduction

The Coronavirus Disease 2019 (COVID-19) pandemic, caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), has resulted in millions of deaths worldwide and represents one of the most extensively documented diseases in modern medical history [1]. To prevent COVID-19, various vaccines were authorized between late 2020 and early 2021, with mRNA vaccines becoming the most widely administered [2]. Substantial interindividual variation in antibody production has been reported following mRNA vaccination, with notable differences observed across racial and geographic populations [3][4]. Factors such as age, sex, underlying health conditions, and vaccine type have been proposed as contributors, along with host genetic factors, including polymorphisms in immune-related genes [3][5-7]. For instance, a study conducted in Germany reported that individuals with the GG genotype of the *IFITM3* polymorphism rs34481144 exhibited lower antibody responses to the BNT162b2 (Pfizer-BioNTech) mRNA vaccine compared to those carrying the A allele [8]. In the United Kingdom, the HLA-DQB1*06:04 allele has also been associated with increased antibody production following SARS-CoV-2 vaccination [9]. These findings have drawn global attention to the influence of genetic background on vaccine-induced immune responses. Numerous human genetic polymorphisms have been shown to affect humoral responses to vaccines targeting common pathogens. These include rs3824949 in tripartite motif-containing 5 (*TRIM5*), which has been associated with anti-rubella antibody responses [10]; rs4792800 in tumor necrosis factor receptor superfamily member 13B (*TNFRSF13B*), linked to IgG levels in healthy individuals [11]; and rs1611350 in human leukocyte antigen-F antisense RNA 1 (*HLA-F-AS1*), associated with variability in immune responses, including IgM, IgG, and IgG1 levels, following vaccination [12]. TRIM5α plays a pivotal role in innate immunity by restricting retroviruses such as human immunodeficiency virus (HIV) [13]. *TNFRSF13B* encodes a protein in the tumor necrosis factor (TNF) receptor superfamily that supports humoral immunity. *HLA-F-AS1* modulates immune responses through interactions with microRNAs.

Given the known racial differences in immune responses, this study investigated the association between three genetic polymorphisms and immune responses to SARS-CoV-2 vaccination in the Japanese population. Anti-SARS-CoV-2 antibody titers were measured in 405 healthy Japanese individuals three weeks after receiving the first dose of an mRNA vaccine, and polymorphisms in *TRIM5*, *TNFRSF13B*, and *HLA-F-AS1* were genotyped in 272 of these participants. In addition, potential associations between antibody titers and various complete blood count (CBC) parameters obtained at the same time point were evaluated. Interindividual variability in vaccine responses remains a major challenge across numerous diseases. By identifying host genetic factors in a Japanese cohort, this study contributes to precision vaccinology and highlights the importance of ethnicity-specific research. Although this study focuses on SARS-CoV-2 mRNA vaccines, the genetic mechanisms identified may also be relevant to other mRNA-based vaccines, highlighting TRIM5α as a potential universal modulator of vaccine-induced immunity.

## II. Materials and methods

### *Ethical Approval and Informed Consent*

All participants, including university employees and students, were recruited from Kanazawa University. All procedures involving human participants were conducted in accordance with the Declaration of Helsinki and institutional guidelines. The study protocol was approved by the Ethics Committee of Kanazawa University (No. 1025-6, August 22, 2022) and Tohoku University (No. 2021-1-1125, March 23, 2022). Written informed consent was obtained from all the participants prior to their inclusion in the study.

### *Study Population and Study Design*

This study included 405 Japanese individuals who received the Moderna mRNA SARS-CoV-2 vaccine. All participants were employees or students from a single institution and were vaccinated as part of a mass vaccination program. International students were excluded from the study. Blood samples were collected approximately three weeks after the first dose, with a median of 21 days (interquartile range [IQR], 19–22), during the period from July 29 to August 20, 2021. Antibody levels and complete blood counts (CBC) were assessed concurrently. Baseline (pre-vaccination) blood samples were not available for analysis.

### *Blood Sample Collection, Anti-SARS-CoV-2 Antibody Titers, and CBC Measurement*

A total of 2 mL of blood was collected in an EDTA-2K tube for CBC analysis using the XE-2000 hematology analyzer (Sysmex Corporation, Kobe, Japan). An additional 9 mL of blood was drawn into a serum separation tube, centrifuged, and the serum was stored at –80℃ until analysis. Measurement of serum antibody titers was outsourced to Sysmex Corporation. Serum levels of anti-

SARS-CoV-2 spike IgG (S-IgG) antibody titers were measured using the HISCL-5000 system with the HISCL™ SARS-CoV-2 S-IgG reagent. S-IgG antibody titers were converted from SU/mL to the World Health Organization (WHO) International Standard BAU/mL (NIBSC code 20/136) using the manufacturer's formula ($y = 5.93x + 0.21$), calibrated against the WHO standard. Nucleocapsid IgG (N-IgG) antibody titers were measured using the HISCL™ SARS-CoV-2 N-IgG reagent, with a cutoff of 10 SU/mL used to confirm prior infection. As reported in the external validation by Noda et al. [14], the performance of the assay was validated using serum samples from 60 individuals with confirmed SARS-CoV-2 infection and 500 pre-pandemic negative controls, yielding an exceptionally high area under the curve (AUC) of 0.9998 and demonstrating excellent stability and reproducibility. These validation samples were not part of the present cohort and were collected and analyzed under independent approvals (NCGM-G-003472-02; NCC 2020-026).

***Extraction of Cell-Free DNA and Genotyping of Genetic Polymorphisms***

Cell-free DNA was extracted from serum samples using the FitAmp Plasma/Serum DNA Isolation Kit (Epigentek Group Inc., Farmingdale, NY, USA). Genetic polymorphisms were analyzed using TaqMan® SNP Genotyping Assays (Thermo Fisher Scientific, Inc., Waltham, MA, USA) with the QuantStudio® 3 Real-Time PCR System (Thermo Fisher Scientific, Inc.). Genotyping was specifically conducted for rs3824949 (Assay ID: C_1452185_20), rs4792800 (Assay ID: C_27968962_10), and rs1611350 (Assay ID: C_26543570_10). These assays were developed and validated by the manufacturer under stringent quality control measures to ensure high accuracy and reproducibility, thereby enhancing the reliability of the genotyping results in this study.

***Statistical Analysis***

Statistical analyses were performed using StatFlex (version 7; Artec Co., Ltd., Osaka, Japan). Variables with $|\text{skewness}| \geq 1.0$ or $|\text{kurtosis}| \geq 3.0$ underwent power transformation. Normality was assessed using the Kolmogorov–Smirnov test ($P$-value > 0.05 = normal; **Supplementary Table 1**). Normally distributed variables were expressed as mean ± standard deviation (SD), while non-normally distributed variables were expressed as median and IQR. For three-group comparisons of non-normal antibody levels, the Kruskal–Wallis test was used; significant results were followed by Dunn's test with Bonferroni correction for pairwise comparisons. Multiple regression was conducted in three steps to identify independent determinants of antibody titers: first, age and CBC parameters (platelet and lymphocyte counts) were included based on preliminary associations; second, genotype (rs3824949) was added to assess its independent effect; finally, a genotype-only model was evaluated. Statistical significance was set at $P$-value < 0.05.

**Table 1** Cross-tabulation analysis of genetic variant genotypes and anti-SARS-CoV-2 antibody levels

| rs3824949 (*TRIM5*) | | GG | GC | CC | H-value | *P*-value |
|---|---|---|---|---|---|---|
| | n (%) | 105 (38.6) | 92 (33.8) | 75 (27.6) | | |
| Ab titer (BAU/mL) | Median | 444 | 380 | 336 | 13.061 | 0.0015 |
| | IQR | 300–664 | 264–608 | 198–412 | | |
| rs4792800 (*TNFRSF13B*) | | AA | AG | GG | H-value | *P*-value |
| | n (%) | 114 (41.9) | 118 (43.4) | 40 (14.7) | | |
| Ab titer (BAU/mL) | Median | 381 | 378 | 368 | 0.580 | 0.7482 |
| | IQR | 268–571 | 267–597 | 232–509 | | |
| rs1611350 (*HLA-F-AS1*) | | TT | TC | CC | H-value | *P*-value |
| | n (%) | 93 (34.2) | 141 (51.8) | 38 (14.0) | | |
| Ab titer (BAU/mL) | Median | 375 | 376 | 387 | 0.255 | 0.8803 |
| | IQR | 260–543 | 258–591 | 272–519 | | |

Data are presented as the number of subjects (n), median antibody titers, and interquartile ranges (IQR) for each genotype of the genetic polymorphisms. Only rs3824949 (*TRIM5*) showed a significant association between genotype and anti-SARS-CoV-2 antibody levels.

Abbreviation: Ab titer, antibody titer; *HLA-F-AS1*, human leukocyte antigen-F antisense RNA 1; IQR, interquartile range; *TNFRSF13B*, tumor necrosis factor receptor superfamily member 13B; *TRIM5*, tripartite motif-containing 5.

## III. Results

### *Genetic Polymorphisms and Anti-SARS-CoV-2 Antibody Titers*

Among the 405 healthy Japanese individuals included in this study, 169 (41.7%) were men and 236 (58.3%) were women, with a median age of 27 (21–45) years (**Supplementary Table 1**). A questionnaire survey conducted at the time of blood collection revealed that none of the participants reported a history of symptomatic SARS-CoV-2 infection during the sample collection period, and no individuals tested positive for SARS-CoV-2 N-IgG. Based on these findings, the immune response due to prior SARS-CoV-2 infection was considered to be minimal in this cohort. We then investigated the association of anti-SARS-CoV-2 antibody titers with genetic variants in genes that were reportedly associated with humoral immune responses to other vaccines. Cell-free DNA samples for these genotyping studies were available in 272 subjects, of whom 96 (35.3%) and 146 (64.7%) were men and women, respectively, with a median age of 23 (21–34) years. The genotype frequencies of the three genetic polymorphisms tested were as follows (**Figure 1A**): rs3824949 (GG = 38.6%, CC = 27.6%, and GC = 33.8%), rs4792800 (AA = 41.9%, GG = 14.7%, and AG = 43.4%), and rs1611350 (TT = 34.2%, CC = 14.0%, and TC = 51.8%). The genotype distribution was consistent with available data on the Japanese population from the HapMap Project and the 1000 Genomes Project. Individuals carrying the GG genotype of the rs3824949 polymorphism had significantly higher antibody levels than those with the CC genotype ($P = 0.0015$; **Table 1**, **Figure 1B**). Specifically, subjects with the GG genotype had the highest levels of antibody titers at 444 (300–664) BAU/mL, followed by the GC genotype at 380 (264–608) BAU/mL and the CC genotype at 336 (198–412) BAU/mL. Meanwhile, rs4792800 and rs1611350 were not associated with the levels of antibodies against SARS-CoV-2 (**Table 1**, **Figures 1C, 1D**).

### *Stratification of Anti-SARS-CoV-2 Antibody Titers and Their Associations with Age, Sex, and CBC Parameters*

Participants were classified into three groups based on antibody titer levels measured three weeks after the first vaccine dose: a high antibody titer group ($\geq$ 550 BAU/mL, n = 112), a moderate antibody titer group (200–549 BAU/mL, n = 227), and a low antibody titer group (< 200 BAU/mL, n = 66). Comparison of demographic factors and CBC parameters among the three groups revealed that younger age was significantly associated with higher antibody titers ($P = 0.00145$). In contrast, no significant difference in antibody titers was observed between men and women. Additionally, higher platelet and lymphocyte counts were significantly correlated with higher antibody titers ($P = 0.01481$ and $P = 0.00750$, respectively; Supplementary **Table 2**).

Next, the Kruskal–Wallis test was performed to conduct multiple comparisons by stratifying age, platelet counts, and lymphocyte counts into five groups. The results indicated that only age and lymphocyte count were significantly associated with anti-SARS-CoV-2 antibody titers ($P = 0.0366$ and $P = 0.0010$, respectively; **Supplementary Table 3**). The group with a platelet count of $\geq 350 \times 10^3/\mu L$ exhibited a clear tendency toward higher antibody titers; however, the difference was not statistically significant due to the limited sample size. Based on these findings, a post hoc analysis using Dunn's test was conducted to further evaluate intergroup differences (**Figure 1E–1G**). The analysis revealed that individuals with lymphocyte counts of 2,500–2,900/μL had significantly higher antibody titers than those in the $\leq$ 1,500/μL, 1,500–1,999/μL, and 2,000–2,499/μL groups ($P < 0.01$, $P < 0.01$, and $P < 0.05$, respectively). In contrast, no significant differences in antibody titers were observed in the $\geq$ 3,000/μL group compared to other groups, likely due to limited sample size and insufficient statistical power. These findings suggest that an increase in lymphocyte count may contribute to an enhanced antibody response (**Figure 1G**).

### *Stepwise Regression Analysis of Determinants of Anti-SARS-CoV-2 Antibody Titers*

To identify factors influencing antibody titers, a multiple regression analysis was performed in three steps (**Table 2**). Covariates were selected based on preliminary statistical analyses (**Supplementary Table 2**), and age, lymphocyte counts, and platelet counts were included as significant predictors. In step 1, a model including age, lymphocyte counts, and platelet counts yielded an $R^2$ of 0.0321. Among these variables, age (Std. $\beta$ = -0.1233, $P = 0.01237$) and lymphocyte counts (Std. $\beta$ = 0.1414, $P = 0.00470$) were significantly associated with antibody titers, indicating that lower age and higher lymphocyte counts were correlated with antibody levels. In contrast, platelet counts showed no significant association ($P = 0.39735$). In Step 2, the addition of genotype (rs3824949) increased the model's $R^2$ to 0.0840 ($\Delta R^2 = 0.0519$), denoting that rs3824949 contributes to the variation in antibody titers. In this model, rs3824949 remained a significant predictor (Std. $\beta$ = 0.1859, $P = 0.00175$), while age also remained significant (Std. $\beta$ = -0.1439, $P$ =

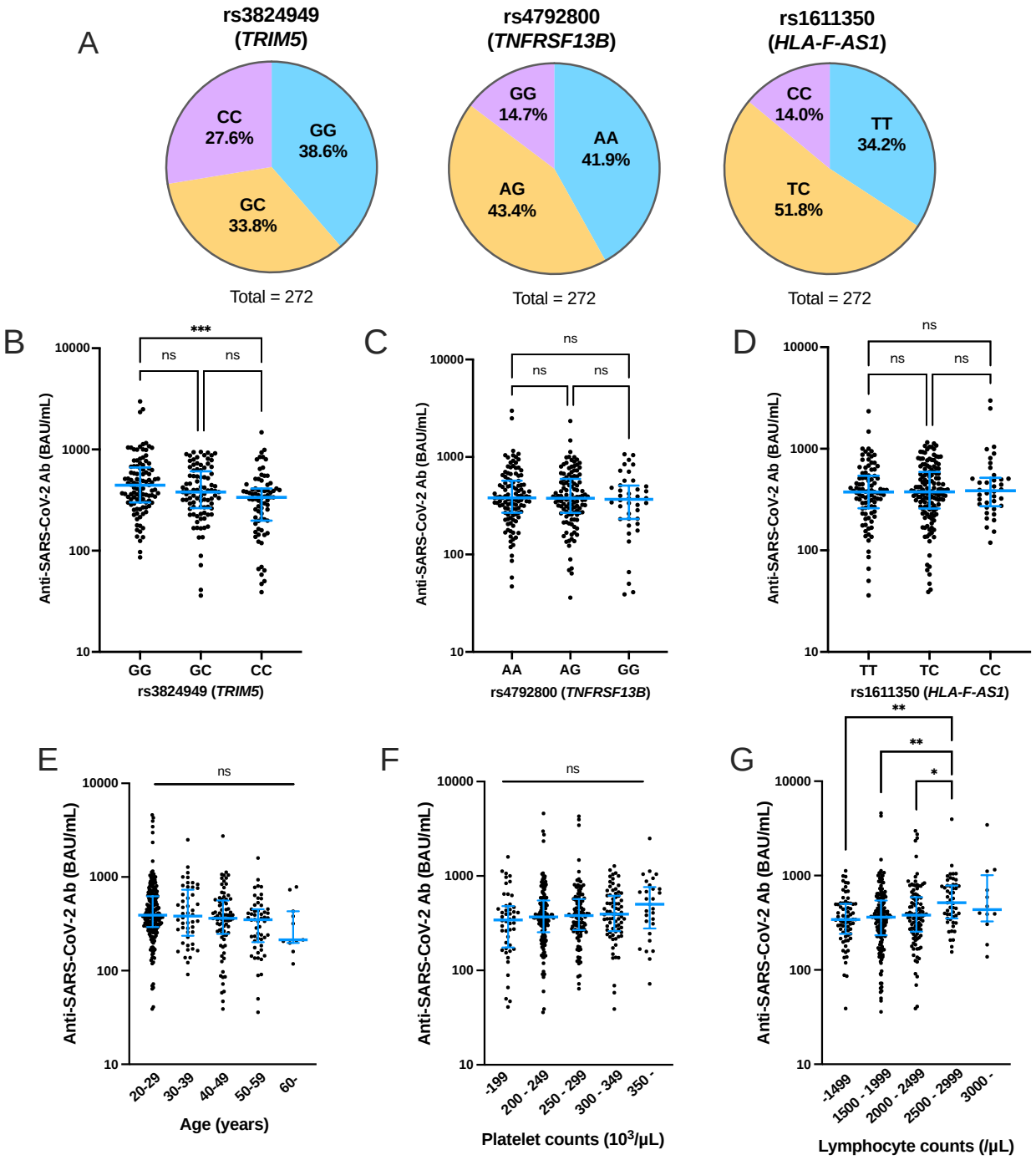


**Figure 1** Distribution of genetic polymorphisms and anti-SARS-CoV-2 antibody titers by genotype and blood cell counts in Japanese individuals

The distribution of genetic polymorphisms (A) and the comparison of anti-SARS-CoV-2 antibody titers among different genotypes and complete blood cell counts are presented. The Kruskal–Wallis test was applied to compare three groups based on anti-SARS-CoV-2 antibody levels, and if a significant difference was detected, Dunn's test with Bonferroni correction was used for pairwise comparisons. All graphs display the median and interquartile range (IQR). (B) Levels of antibody titers in relation to the rs3824949 (*TRIM5*) genotypes (GG, GC, CC). The GG genotype is related to higher levels of antibody titers compared with the CC genotype (***$P = 0.0009$, Dunn's test with Bonferroni correction). (C) Levels of antibody titers in relation to the rs4792800 (*TNFRSF13B*) genotypes (AA, AG, GG). No significant difference in levels of antibody titers was observed across the genotypes ($P = 0.7482$). (D) Levels of antibody titers in relation to the rs1611350 (*HLA-F-AS1*) genotypes (TT, TC, CC) showed no significant association ($P = 0.8803$). (E) Age, (F) platelet counts, and (G) lymphocyte counts were classified into five groups, showing the respective anti-SARS-CoV-2 antibody titers. In the graph of (G), *$P < 0.05$, **$P < 0.01$. All graphs show the median and interquartile range (IQR).

Abbreviation: *HLA-F-AS1*, human leukocyte antigen-F antisense RNA 1; IQR, interquartile range; *TNFRSF13B*, tumor necrosis factor receptor superfamily member 13B; *TRIM5*, tripartite motif-containing 5.

**Table 2** Multivariate regression analysis for anti-SARS-CoV-2 antibody titers, including age, lymphocyte counts, and platelet counts with or without genotype (rs3824949)

| Model | Variable included | Unstd. Coef. β | Std. Error | Std. β | t-val | *P*-value | $R^2$ | $\Delta R^2$ |
|---|---|---|---|---|---|---|---|---|
| 1 | (Constant) | -1941.1 | 880 | — | — | — | 0.0321 | — |
| | Age | -4.633 | 1.844 | -0.1233 | -2.5129 | 0.01237 | | |
| | LYM | 270.4 | 95.12 | 0.1414 | 2.8246 | 0.00470 | | |
| | PLT | 98.26 | 116.0 | 0.0422 | 0.8473 | 0.39735 | | |
| 2 | (Constant) | -1625.1 | 737.2 | — | — | — | 0.0840 | 0.0519 |
| | Age | -4.276 | 1.737 | -0.1439 | -2.4621 | 0.01444 | | |
| | LYM | 150.9 | 80.62 | 0.1113 | 1.8714 | 0.06238 | | |
| | PLT | 176.6 | 98.28 | 0.1067 | 1.7965 | 0.07355 | | |
| | rs3824949 | 78.59 | 24.86 | 0.1859 | 3.1614 | 0.00175 | | |
| 3 | (Constant) | 355.2 | 34.42 | - | - | - | 0.0448 | - |
| | rs3824949 | 92.93 | 25.09 | 0.2199 | 3.7039 | 0.00026 | | |

The GG genotype of rs3824949 and age were identified as significant determinants of anti-SARS-CoV-2 antibody titers. Platelet and lymphocyte count showed weak associations. The inclusion of rs3824949 in the regression model significantly improved the explanatory power, as indicated by an increase in $R^2$ from 0.0321 to 0.0840 ($\Delta R^2 = 0.0519$). A genotype-only model yielded an $R^2$ of 0.0448, suggesting that while rs3824949 contributes independently to antibody titer variation, its explanatory power is enhanced when combined with demographic and hematological variables. Abbreviation: LYM, lymphocyte counts; PLT, platelet counts; Std. β, standardized coefficient beta; Std. Error, standard error; Unstd. Coef. β, unstandardized coefficient beta.

0.01444). However, the effect of lymphocyte counts was attenuated ($P = 0.06238$), and platelet counts remained non-significant ($P = 0.07355$). In Step 3, a genotype-only model was constructed, yielding an $R^2$ of 0.0448, with rs3824949 remaining a significant predictor (Std. β = 0.2199, $P = 0.00026$), highlighting the strength of its effect. These results indicate that the rs3824949 genotype makes a significant and independent contribution to the variation in antibody titers. However, $R^2$ was lower compared to the model incorporating covariates (Step 2).

## IV. Discussion

In this study, we confirmed the substantial inter-individual variability in SARS-CoV-2 antibody titers and categorized participants into high, moderate, and low responders. A higher frequency of the G allele of rs3824949 in the *TRIM5* gene was significantly associated with enhanced antibody response following Moderna vaccination ($P = 0.0015$). In contrast, rs4792800 in the *TNFRSF13B* and rs1611350 in the *HLA-F-AS1* showed no significant association with antibody levels. These findings suggest that host genetic factors may contribute to SARS-CoV-2 mRNA vaccination in the Japanese population.

The *TRIM5* gene encodes tripartite motif-containing protein 5 alpha, also known as RING finger protein 88, a restriction factor that targets retroviral capsids and plays a regulatory role in innate immunity [15,16]. TRIM5α has been proposed to function as a pattern recognition receptor (PRR) involved in pathogen recognition [17]. The rs3824949 polymorphism identified in this study is located in the 5' UTR, which may influence *TRIM5* gene expression [18], although the precise mechanisms by which rs3824949 affects the antibody response remain unclear. In this study, the G allele of rs3824949 was associated with enhanced immune response to SARS-CoV-2 vaccination. The allele frequency in the Japanese population (0.543) is comparable to that observed in European populations (0.442) and is similar to the global average (0.495), suggesting that the immunogenetic impact of rs3824949 may be relevant across diverse populations. This supports the generalizability of our findings and highlights the potential utility of *TRIM5* genotyping in broader vaccine response studies. Given that TRIM5α functions as a restriction factor against various viruses, its role may extend beyond mRNA vaccines to other vaccine platforms. Future studies should investigate the impact of TRIM5α across diverse vaccine modalities, including viral vector, protein-based, and inactivated vaccines, and elucidate the molecular mechanisms through which rs3824949 modulates immune responses.

As a secondary analysis, we examined demographic and CBC parameters potentially associated with antibody titers following the first dose of an anti-SARS-CoV-2 mRNA vaccine. Age, lymphocyte counts, and platelet count were independently and significantly associat-

ed with antibody titers, with higher titers observed in younger individuals and those with elevated lymphocyte and platelet counts. These findings are consistent with previous studies [8),19)-21)]. Notably, even after adjustment for these variables, the rs3824949 polymorphism remained significantly associated with higher antibody titers (**Table 2**). Furthermore, the model incorporating age, lymphocyte counts, and platelet counts exhibited greater explanatory power compared to the genotype-only model (**Table 2**; $\Delta R^2 = 0.0519$). These results suggest that the effect of rs3824949 may be mediated, at least in part, through interactions with age and hematologic parameters.

In this study, lymphocyte and platelet counts measured post-vaccination were positively associated with antibody titers. However, because CBC parameters were obtained at a single time point after immunization and pre-vaccination baselines were unavailable, these findings should be interpreted as post-vaccination correlates that may reflect transient reactogenicity [22)], rather than as causal determinants of antibody responses. Notably, the association of rs3824949 with higher titers remained significant after adjustment (**Table 2**), indicating an effect at least partially independent of hematologic correlates. Platelets have been reported to modulate immune function through interactions with immune cells and may contribute to B cell activation [23)–25)]; however, we did not investigate this mechanism in the present study. Furthermore, GWAS have shown that the C allele of rs3824949 is associated with lower platelet counts ($\beta = -0.0177$, $P = 2 \times 10^{-20}$) [26)], suggesting that interindividual variation in platelet levels may be genetically modulated. However, whether platelet counts mediate the association between rs3824949 and antibody titers cannot be determined from our cross-sectional study design. Prospective longitudinal measurements before and after vaccination, along with mediation analyses, will be required to clarify the causal relationship and underlying mechanisms.

Although the COVID-19 pandemic has transitioned to endemicity, the insights from mRNA vaccine genetics remain broadly relevant. The role of TRIM5α in innate immunity suggests its potential relevance not only to current but also to future mRNA vaccines, including those targeting influenza, HIV, or Zika virus. This positions TRIM5α as a promising target for enhancing vaccine design. The observed association between rs3824949 and antibody responses further highlights the therapeutic potential of modulating TRIM5α to improve vaccine efficacy in low responders, potentially extending to mRNA-based cancer immunotherapies. As global health systems increasingly embrace personalized medicine, screening for genetic variants such as rs3824949 could facilitate risk stratification and individualized vaccination strategies. Immunization schedules tailored to genetic profiles may optimize vaccine-induced protection in high-risk or genetically susceptible populations. Future research should explore whether *TRIM5* polymorphisms influence immune responses to other vaccine platforms and their durability following booster doses or in the context of hybrid immunity. These insights may inform both vaccine development and public health strategies.

This study has several limitations. First, the positivity rate of N-IgG antibody titers measured using the HISCL™ SARS-CoV-2 N-IgG reagent reportedly declines to 26.5% more than three months after SARS-CoV-2 infection [27)]. Therefore, some participants may have experienced asymptomatic SARS-CoV-2 infections that were not detected by N-IgG testing, potentially influencing their post-vaccination immune responses [28)]. However, this effect could not be assessed in the present study. Second, since the analysis focused on antibody responses after the first vaccine dose, it remains unclear whether rs3824949 or other polymorphisms affect responses to the second or subsequent doses. Third, CBC parameters were measured only once, approximately three weeks after the first dose, without pre-vaccination baseline values. Therefore, transient post-vaccination changes cannot be distinguished from stable genotype-related differences. In addition, this cross-sectional analysis cannot determine whether platelet or lymphocyte counts mediate the association between rs3824949 and antibody responses; prospective longitudinal data are required. Finally, this study did not assess whether these genetic variations, including rs3824949, are associated with vaccine-related adverse events in the Japanese population.

In conclusion, the immune response to the Moderna vaccine in the Japanese population varied due to the genetic influence of the *TRIM5* polymorphism rs3824949, with individuals carrying the GG genotype exhibiting significantly higher antibody titers. Although the urgency of COVID-19 research has diminished, our findings address enduring questions regarding host–pathogen interactions and genetic variability in vaccine responses. By extending beyond the context of SARS-CoV-2, this study provides a foundation for optimizing mRNA vaccines across diverse populations and disease targets.

**Supplementary Materials**

Supplementary Tables 1, 2, and 3 provide additional data relevant to this study and are available online.

**Funding**

This research was supported by AMED under Grant Number JP22ek0210154.

**Conflicts of Interest**

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**Acknowledgments**

Some of the results were obtained in collaborative work with Sysmex Corporation. The authors sincerely appreciate the invaluable assistance of Haruka Moriya, Tomoki Togashi, Mie Morikawa, and Takamasa Katagiri from Kanazawa University, as well as the support provided by the staff of the Ishikawa Medical Association Clinical Laboratory Center in sample preparation. Additionally, the authors acknowledge Enago (www.enago.jp) for the English language review.

**Authorship Contributions**

Conceptualization: J. Luis Espinoza; Methodology: J. Luis Espinoza; Formal analysis and investigation: J. Luis Espinoza, Satomi Nagaya, Nguyen Hoang Thao Giang, Koichi Yamaguchi, and Kota Goto; Writing-original draft preparation: Satomi Nagaya, Nguyen Hoang Thao Giang, and J. Luis Espinoza; Writing-review and editing: J. Luis Espinoza, Hisanori Horiuchi, and Eriko Morishita; Funding acquisition: Hisanori Horiuchi and Eriko Morishita; Resources: Satomi Nagaya, J. Luis Espinoza, and Eriko Morishita; Supervision: Hisanori Horiuchi and Eriko Morishita.

**Disclosure of Conflicts of Interest**

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**References**

1) Msemburi W, Karlinsky A, Knutson V, et al. The WHO estimates of excess mortality associated with the COVID-19 pandemic. Nature. 2023; 613 (7942): 130-7. doi: 10.1038/s41586-022-05522-2.

2) Graña C, Ghosn L, Evrenoglou T, et al. Efficacy and safety of COVID-19 vaccines. Cochrane Database Syst Rev. 2022;12 (12): CD015477. doi: 10.1002/14651858.CD015477.

3) Quer G, Gadaleta M, Radin JM, et al. Inter-individual variation in objective measure of reactogenicity following COVID-19 vaccination via smartwatches and fitness bands. NPJ Digit Med. 2022; 5 (1): 49. doi: 10.1038/s41746-022-00591-z.

4) Krüttgen A, Haase G, Haefner H, et al. Large inter-individual variability of cellular and humoral immunological responses to mRNA-1273 (Moderna) vaccination against SARS-CoV-2 in healthcare workers. Clin Exp Vaccine Res. 2022; 11(1): 96-103. doi: 10.7774/cevr.2022.11.1.96.

5) Pereira NL, Ahmad F, Byku M, et al. COVID-19: Understanding inter-individual variability and implications for precision medicine. Mayo Clin Proc.2021; 96 (2): 446-63. doi: 10.1016/j.mayocp.2020.11.024.

6) Glück V, Grobecker S, Köstler J, et al. Immunity after COVID-19 and vaccination: Follow-up study over 1 year among medical personnel. Infection. 2022; 50 (2): 439-46. doi: 10.1007/s15010-021-01703-9.

7) Mentzer AJ, O'Connor D, Bibi S, et al. Human leukocyte antigen alleles associate with COVID-19 vaccine immunogenicity and risk of breakthrough infection. Nat Med. 2023; 29(1): 147-57. doi: 10.1038/s41591-022-02078-6.

8) Čiučiulkaitė I, Siffert W, Elsner C, et al. Influence of the single nucleotide polymorphisms rs12252 and rs34481144 in *IFITM3* on the antibody response after vaccination against COVID-19. Vaccines (Basel). 2023; 11 (7): 1257. doi: 10.3390/vaccines11071257.

9) Xie J, Mothe B, Alcalde Herraiz M, et al. Relationship between HLA genetic variations, COVID-19 vaccine antibody response, and risk of breakthrough outcomes. Nat Commun. 2024; 15(1):4031. doi: 10.1038/s41467-024-48339-5.

10) Haralambieva IH, Ovsyannikova IG, Pankratz VS, et al. The genetic basis for interindividual immune response variation to measles vaccine: New understanding and new vaccine approaches. Expert Rev Vaccines. 2013; 12(1): 57-70. doi: 10.1586/erv.12.134.

11) Liao M, Ye F, Zhang B, et al. Genome-wide association study identifies common variants at TNFRSF13B associated with IgG level in a healthy Chinese male population. Genes Immun. 2012; 13(6): 509-13. doi: 10.1038/gene.2012.26.

12) Yucesoy B, Talzhanov Y, Johnson VJ, et al. Genetic variants within the MHC region are associated with immune responsiveness to childhood vaccinations. Vaccine. 2013; 31(46): 5381-91. doi: 10.1016/j.vaccine.2013.09.026.

13) Carthagena L, Bergamaschi A, Luna JM, et al. Human TRIM gene expression in response to interferons. PLoS One. 2009; 4(3): e4894. doi: 10.1371/journal.pone.0004894.

14) Noda K, Matsuda K, Yagishita S, et al. A novel highly quantitative and reproducible assay for the detection of anti-SARS-CoV-2 IgG and IgM antibodies. Sci Rep. 2021; 11(1): 5198. doi: 10.1038/s41598-021-84387-3.

15) Pertel T, Hausmann S, Morger D, et al. TRIM5 is an innate

immune sensor for the retrovirus capsid lattice. Nature. 2011; 472(7343): 361-5. doi: 10.1038/nature09976.

16) Nakayama EE, Shioda T. Role of human TRIM5α in intrinsic immunity. Front Microbiol. 2012; 3: 97. doi: 10.3389/fmicb.2012.00097.

17) Ishii KJ, Koyama S, Nakagawa A, et al. Host innate immune receptors and beyond: Making sense of microbial infections. Cell Host Microbe. 2008; 3(6): 352-63. doi: 10.1016/j.chom.2008.05.003.

18) Leppek K, Das R, Barna M. Functional 5' UTR mRNA structures in eukaryotic translation regulation and how to find them. Nat Rev Mol Cell Biol. 2018; 19 (3): 158-74. doi: 10.1038/nrm.2017.103.

19) Hussein K, Dabaja-Younis H, Szwarcwort-Cohen M, et al. Third BNT162b2 vaccine booster dose against SARS-CoV-2-induced antibody response among healthcare workers. Vaccines (Basel). 2022; 10 (10): 1741. doi: 10.3390/vaccines10101741.

20) Pellini R, Venuti A, Pimpinelli F, et al. Initial observations on age, gender, BMI and hypertension in antibody responses to SARS-CoV-2 BNT162b2 vaccine. EClinicalMedicine. 2021; 36: 100928. doi: 10.1016/j.eclinm.2021.100928.

21) Ruggeri EM, Nelli F, Giannarelli D, et al. Dynamic changes in peripheral lymphocytes and antibody response following a third dose of SARS-CoV-2 mRNA-BNT162b2 vaccine in cancer patients. Sci Rep. 2022; 12(1): 21908. doi: 10.1038/s41598-022-25558-8.

22) Krüttgen A, Haase G, Haefner H, et al. Large inter-individual variability of cellular and humoral immunological responses to mRNA-1273 (Moderna) vaccination against SARS-CoV-2 in health care workers. Clin Exp Vaccine Res. 2022; 11(1): 96-103. doi: 10.7774/cevr.2022.11.1.96.

23) Scherlinger M, Richez C, Tsokos GC, et al. The role of platelets in immune-mediated inflammatory diseases. Nat Rev Immunol. 2023; 23(8): 495-510. doi: 10.1038/s41577-023-00834-4.

24) Sun W, Zhu C, Li Y, et al. B cell activation and autoantibody production in autoimmune diseases. Best Pract Res Clin Rheumatol. 2024; 38(2): 101936. doi: 10.1016/j.berh.2024.101936.

25) Cognasse F, Hamzeh-Cognasse H, Lafarge S, et al. Human platelets can activate peripheral blood B cells and increase production of immunoglobulins. Exp Hematol. 2007; 35(9): 1376-87. doi: 10.1016/j.exphem.2007.05.021.

26) Sakaue S, Kanai M, Tanigawa Y, et al. A cross-population atlas of genetic associations for 220 human phenotypes. Nat Genet. 2021; 53(10): 1415-24. doi: 10.1038/s41588-021-00931-x.

27) Ouoba S, Sugiyama A, Ko K, et al. Development of a unit conversion tool for five quantitative anti-spike assays and agreement analysis of three qualitative anti-nucleocapsid assays for SARS-CoV-2. J Med Virol. 2024; 96(8): e29826. doi: 10.1002/jmv.29826.

28) Reynolds CJ, Pade C, Gibbons JM, et al. Prior SARS-CoV-2 infection rescues B and T cell responses to variants after first vaccine dose. Science. 2021; 372(6549): 1418-23. doi: 10.1126/science.abh1282.