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Comparison of IL-6 measurement methods with a special emphasis on COVID-19 patients according to equipment and sample type

Yu Jeong Choi[1] | Juhye Roh[2] | Sinyoung Kim[1] | Kyung-A Lee[1] | Younhee Park[1] 🟢

[1]Department of Laboratory Medicine, Severance Hospital, Yonsei University College of Medicine, Seoul, Korea

[2]Department of Laboratory Medicine, Hallym University Sacred Heart Hospital, Anyang, Korea

Correspondence
Younhee Park, Department of Laboratory Medicine, Severance Hospital, Yonsei University College of Medicine, 50-1, Yonsei-ro, Seodaemun-gu, Seoul, Korea.
Email: younheep@yuhs.ac

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Abstract

Background: Interleukin-6 (IL-6) is a multifunctional cytokine associated with various diseases, including coronavirus disease (COVID-19). Although IL-6 levels can be assessed using serum samples, use of the AFIAS (Boditech Med Inc.) automated immunoassay analyzer enables quick and simple measurement of IL-6 levels in both serum and whole blood specimens. This study aimed to assess the correlation between IL-6 measurements obtained from the AFIAS IL-6 assay and Elecsys IL-6 assay (Roche Diagnostics). Additionally, utilization of the AFIAS IL-6 assay was evaluated.

Methods: The IL-6 levels from 113 serum samples quantified using two assay systems were evaluated for their degree of correlation. Meanwhile, the linearity, analytical sensitivity, and precision/reproducibility of the AFIAS IL-6 assay were also assessed.

Results: Quantification of IL-6 with the AFIAS IL-6 and Elecsys IL-6 assays showed excellent agreement (kappa 0.802) and were found to be correlated (y = −0.2781 + 1.068x; 95% confidence interval: 1.007–1.124). AFIAS IL-6 showed good analytical performances. IL-6 levels were significantly higher in deceased patients compared to those with non-complicated disease and those who were intubated ($p = 0.002$ and $p < 0.0001$, respectively). Finally, IL-6 levels more accurately predicted poor prognosis in patients, than did C-reactive protein (area under the curve, 0.716 vs. 0.634).

Conclusion: The overall analytical performance of the AFIAS assay was comparable to that of the Elecsys IL-6 assay. In light of the ongoing COVID-19 pandemic, the AFIAS may be an attractive tool for measuring IL-6 levels.

KEYWORDS
AFIAS, analytical performance, coronavirus disease 2019, cytokines, Elecsys, fluorescence immunoassay, interleukin-6

1 | INTRODUCTION

Interleukin-6 (IL-6) is a multifunctional cytokine, associated with a range of diseases and medical conditions. IL-6 is a single chain phosphorylated glycoprotein composed of 184 amino acids and has a molecular weight of 22–27 kDa.[1] It forms a complex with the IL-6 receptor (IL-6R) and glycoprotein 130 (gp130) to activate numerous signaling pathways, such as the JAK/STAT3, Ras/MAPK,

and PI3K-PKB/Akt pathways.[2] Abnormal activation of these pathways leads to various pathologic conditions including cancer, multiple sclerosis, rheumatoid arthritis, diabetes, anemia, inflammatory bowel disease, Crohn's disease, and Alzheimer's disease.[3] IL-6 is an established therapeutic target in some of these pathologies, thereby playing an important role in their management.[4–8] In addition, IL-6 production is elevated in acute inflammatory reactions, which are associated with trauma and infection.[1] IL-6 levels can therefore be used to predict future complications or detect missed injuries in trauma patients.[9,10] Additionally, sequential measurement of IL-6 in patients admitted to the intensive care unit (ICU) can be useful for evaluating the severity of systemic inflammatory response syndrome (SIRS), sepsis, and septic shock.[11,12]

Recent studies have revealed that IL-6 is also related to the prognosis of coronavirus disease (COVID-19).[13] Coronaviruses can cause acute respiratory distress syndrome (ARDS) via excessive activation and dysregulation of host immune responses, such as the cytokine release syndrome.[14,15] Reports comparing IL-6 levels between patients with complicated disease (ARDS, ICU admission) and non-complicated disease revealed that the IL-6 level was significantly elevated in the former group compared to that in the latter group.[16–21] In fact, one study showed that baseline IL-6 levels were positively correlated with bilateral pulmonary involvement and maximum body temperature.[18] Furthermore, IL-6 levels in patients after receiving treatment were significantly reduced as their pulmonary computed tomography images showed improvement, whereas patients experiencing clinical deterioration had increased IL-6 levels. Another study analyzing the risk factors for ARDS and death in patients with COVID-19 observed elevated IL-6 levels associated with both ARDS and death.[21]

These circumstances necessitate a method for measuring IL-6 levels in a simple and timely manner. The AFIAS IL-6 assay (Boditech Med Inc.) is a fluorescence immunoassay (FIA) for the quantitative detection of IL-6 in not only serum and plasma samples but also in whole blood samples. The assay does not require strict maintenance and comprises a simple two-step process, eliminating the need for highly trained personnel to run the protocol. Moreover, the size of the instrument is smaller than that used in conventional methods of measurement, such as the Elecsys IL-6 assay (Roche Diagnostics), enabling potential use at outpatient clinics and in small-sized laboratories. The more conventional Elecsys IL-6 assay is an electro-chemiluminescence immunoassay (ECLIA) that can analyze serum and plasma samples, however, cannot process whole blood samples; moreover, the machine is quite large and requires strict maintenance.

This study aimed to compare the analytical performance of the AFIAS IL-6 assay with that of the Elecsys IL-6 assay system by evaluating its correlation. We hypothesize that the IL-6 measurement obtained using the AFIAS IL-6 assay will correlate well with that obtained with Elecsys IL-6 assay, making the AFIAS IL-6 assay a suitable alternative. We also hypothesize that IL-6 levels measured in serum and whole blood will correlate sufficiently such that the two types of samples may be used interchangeably.

TABLE 1 Patient demographics

Variables	Patients (n = 34)
Age, years	
Mean (SD)	60.0 (19.9)
Sex, n (%)	
Male	19 (55.9)
Female	15 (44.1)
In-patient	30 (88.2)
Out-patient	4 (11.8)
Main diagnosis, n (%)	
Alström syndrome	1 (2.9)
Coronavirus disease 2019	23 (67.6)
Ependymoma	1 (2.9)
Glioblastoma	1 (2.9)
Lung cancer (adenocarcinoma)	2 (5.9)
Myasthenia gravis	3 (8.8)
Pituitary carcinoma	1 (2.9)
Juvenile idiopathic arthritis	2 (5.9)

2 | MATERIALS AND METHODS

2.1 | Sample collection and comparison study

A total of 113 serum samples from 34 patients, collected during January 2021, were analyzed. An additional 40 whole blood samples were simultaneously collected from these patients. Patient demographics are shown in Table 1. The mean age of the patients was 60 years (range: 11–84 years). The male to female ratio was 1.27:1. Twenty-three (67.6%) patients were diagnosed with COVID-19, from whom 99 samples were collected.

Serum samples were collected in SST tubes (AB Medical; REF 321502) and centrifuged at 3,500 rpm for 10 min before analysis. Whole blood samples were collected in ethylenediaminetetraacetic acid (EDTA) tubes (AB Medical; REF 410303) and analyzed without further processing. Blood samples were drawn regardless of specific time or fasting state. IL-6 levels were quantified on the same day that the samples were collected using both the Elecsys IL-6 (Cobas e602 analyzer) and AFIAS IL-6 assays according to the manufacturers' recommendations. The Elecsys IL-6 assay utilized the ECLIA method, whereas the AFIAS IL-6 assay used the FIA method. Unlike Elecsys IL-6, AFIAS IL-6 included EDTA whole blood as a recommended specimen. Moreover, the Elecsys assay used a fully automated online analyzer, while AFIAS assay used a partially automated offline analyzer. The duration of the assays was 18 and 12 min for Elecsys IL-6 and AFIAS IL-6, respectively. Their analytical performance data, as presented by the manufacturers, are summarized in Table 2. Results not quantified by either assay were excluded from the correlation analysis.[22]

This research was conducted in accordance with all relevant national regulations, institutional policies, and the tenets of the

Declaration of Helsinki (as revised in 2013). The study was approved by the Institutional Review Board of Severance Hospital, Seoul, Korea (IRB No. 4–2020–1246). The requirement for informed consent was waived due to the retrospective nature of this study, provided that all patient data were anonymized, and the study involved samples that were already available rather than those collected prospectively.

2.2 | AFIAS IL-6 analytical performance

Positive controls at different concentrations (9.0 pg/ml, 42.61 pg/ml, and 1,274.0 pg/ml) were provided by the manufacturer and tested in duplicates twice per day for 21 days. Using this data, the inter- and intra-assay precision were evaluated. The acceptance criterion was set to a coefficient of variation (CV%) <10%.[23]

To verify the linearity of the AFIAS IL-6 assay, two serum samples with low (2 pg/ml) and high concentrations (2,039 pg/ml) were mixed in the following proportions to produce five concentrations: 4:0, 3:1, 2:2, 1:3, and 0:4. The high and low concentrations were verified using Elecsys IL-6 assay. The five concentrations were measured in duplicate.[24] The allowable error criteria were set at ≤10% residual, except for the lowest level.

To assess the analytical sensitivity of the AFIAS IL-6 assay, calibrators with appropriate concentrations of 2 pg/ml and 9 pg/ml, as well as the assay buffer were used. The assay buffer was tested 8 times per day for 3 days using three different lots. Rank position formula was used for the limit of blank (LOB) calculation (Rank position = $(72 \times 0.95) + 0.5$), and the LOB was selected as the highest values obtained from all lots. The results were then sorted in ascending order,

and the 68th and 69th results were selected according to formula (LOB = Rank_{68} value +0.5 X (Rank_{69} value – Rank_{68} value). Next, the limit of detection (LOD) was determined by creating four mixtures with the following calibration solution (2 pg/ml): diluent (Gibco. No. 26050–088) proportions: 1:3, 1:1, 1:0.33, and 1:0. Each solution was run five times on 3 days using three different lots. Additionally, the limit of quantitation (LOQ) was determined by mixing 1.5, 1.8, 2, 2.3, and 2.5 pg/ml materials with 9 pg/ml calibrator and diluent (Gibco). These analyses were performed four times on three different days with three different lots. LOQ was set as the lowest concentration with <20% of total allowable error (TE).

2.3 | IL-6 values relative to clinical condition of patients

To determine whether any significant differences in IL-6 values occurred based on the condition of the patient, samples were divided into the following three groups: samples obtained from those with non-complicated disease, those requiring ventilation, and deceased patients. IL-6 and C-reactive protein (CRP) levels of samples collected on the same day were compared. CRP levels were tested using the Cobas8000 system (Roche). Samples with IL-6 levels below the lower limit of detection (LLOD; 2.0 pg/ml) were excluded.

2.4 | Statistical analysis

For statistical analysis, Microsoft Excel 2013 (Microsoft), Analyse-it for Microsoft Excel Method Evaluation Edition version 5.40.2 (Analyse-it Software, Ltd.), IBM SPSS Statistics v.23 (IBM Corp.), and Prism 8.0 (GraphPad Software, Inc) were used. Passing-Bablok regression, Bland-Altman analysis, and Cohen's kappa coefficients were used to evaluate the correlation and agreement between the two assays. Kappa (k) values >0.60 indicate substantial to perfect agreement, while values between 0.20 and 0.60 suggest fair to moderate agreement, and values <0.20 indicate poor to slight agreement.[25] Linearity was evaluated using linear fit regression. The Mann-Whitney U test was used to evaluate whether there were any significant differences in the distribution of IL-6 measurements according to the clinical severity of the patients. p values <0.05 were considered statistically significant.

TABLE 2 Analytical performance characteristics of Elecsys IL-6 and AFIAS IL-6

	Elecsys IL–6	AFIAS IL–6
Limit of blank (LOB)	1.0 pg/ml	0.5 pg/ml
Limit of detection (LOD)	1.5 pg/ml	1.0 pg/ml
Limit of quantitation (LOQ)	2.5 pg/ml	2.0 pg/ml
Linear range	1.5–5,000 pg/ml	2.0–2,500 pg/ml
Reference range	~7.0 pg/ml	~7.0 pg/ml

TABLE 3 Within-run, within-laboratory, between-lot, between-person, and between-site imprecision as recommended by the Clinical and Laboratory Standard Institute (CLSI EP5-A3)

| Conc. | Repeatability | | Total precision | | Lot to lot precision | | Between person | | Between site | |
| | (Within-Run) | | (Within-laboratory precision) | | | | | | | |
[pg/ml]	AVG	CV (%)	AVG	CV (%)	AVG	CV (%)	AVG	CV (%)	AVG	CV (%)
9	8.97	6.1	8.93	6.1	8.96	6.3	8.82	7.3	8.97	6.3
42.61	41.91	5.4	42.3	6	42.58	6.2	41.42	7.2	42.15	7.4
1,274	1,254.8	6.5	1,245.2	6.2	1,265.7	6.5	1,234	7.5	1,264.8	5.6

3 | RESULTS

3.1 | Analytical performance

The results of the 68th and 69th tested samples from each lot were 0.49, 0.50, 0.50, 0.50, 0.50, and 0.50, respectively. Thus, the calculated LOB was set to 0.50, while the LOD was selected as 1.0 pg/ml based on the 1.00, 0.95, and 0.97 values obtained for the three different lots. The IL-6 concentrations within 20% TE were 2.0, 2.3, and 2.5 pg/ml; as such, the LOQ was set to 2.0 pg/ml (Table 2). The within-run, within-laboratory, between-lot, between-person, and between-site imprecision values are summarized in Table 3. All % CVs were within 10%, indicating good assay precision.

The linear range of the AFIAS IL-6 assay was 2–2,039 pg/ml. The linearity of the quantitation assessed was excellent with an R^2 value of 0.995 (Figure 1). The equation for the linear regression line was $y = 1.0167x + 11.04$. The measurement values and regression analysis results are shown on Table S1.

3.2 | Comparison

The comparison between the Elecsys IL-6 and AFIAS IL-6 assays is shown in Figure 2. Of the 113 samples collected, 104 samples were quantified using both assays. The values quantified with the Elecsys assay ranged from 1.7 to 1,198 pg/ml (median 51.7 pg/ml, interquartile range (IQR) 96.3 pg/ml), while those quantified with the AFIAS IL-6 assay ranged from 2.25 to 1,005.61 pg/ml (median 32.0 pg/ml, IQR 70.6 pg/ml). The IL-6 results of both assays were strongly correlated with each other ($r = 0.967$, Figure 2A). The regression line
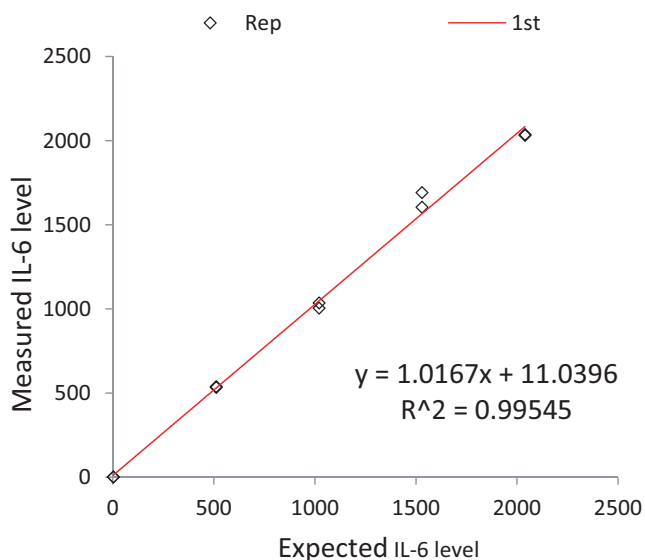

FIGURE 1 Linearity of AFIAS IL-6. The linear range of AFIAS IL-6 demonstrated in this study was 2–2,039 pg/ml. The equation for the linear regression line was $y = 1.0167x + 11.04$, and its determination coefficient was 0.9962

was $y = -0.2781+1.068x$ (95% confidence interval [CI] of the slope: 1.007–1.124, 95% CI of the y-intercept: −0.3913– −0.1549). Bland-Altman analysis indicated that the mean difference in the titer (AFIAS − Elecsys) was −0.167 Log pg/ml (mean bias of −34.26%; Figure 2B). The comparison between serum and whole blood IL-6 levels measured using the AFIAS assay is shown in Figure S1. Of the 40 samples collected, 34 were quantified and analyzed as both serum and whole blood. The regression line was $y = 0.05064 + 0.9647x$ (95% CI of the slope: 0.877–1.077, 95% CI of the y-intercept: −0.1489–0.2197), and the correlation coefficient was 0.978. The average bias between the two types of samples was 0.024 Log pg/ml (5.14%) (whole blood − serum), with no specific trend for increasing IL-6 levels.

The categorical distribution of all serum samples according to the cut-off value of each assay (7 pg/ml) is summarized in Table 4. Agreement between qualitative results for Elecsys and AFIAS was 92.92% (105/113), and the Cohen's kappa coefficient was 0.802 (95% CI: 0.736–0.868). There were eight discrepant samples, in which the Elecsys showed measurements higher than the cut-off value while the AFIAS showed measurements lower than the cut-off value. The IL-6 values in these eight samples ranged from 8.7 to 12.5 (mean 10.8) pg/ml when measured with Elecsys, and from 2.25 to 6.31 (mean 5.1) pg/ml when measured with the AFIAS. All the samples were within 5 pg/ml of the cut-off value.

3.3 | IL-6 values relative to the clinical condition of the patient

Median IL-6 levels in samples from patients with non-complicated disease ($n = 22$), those requiring ventilation ($n = 69$), and deceased patients ($n = 22$) were 1.171 Log pg/ml (IQR: 1.20 Log pg/ml), 1.53 Log pg/ml (IQR: 0.54 Log pg/ml), and 1.89 Log pg/ml (IQR: 0.62 Log pg/ml), respectively (Figure 3A). IL-6 levels in deceased patients were significantly higher than in those with non-complicated disease and in those who were intubated ($p = 0.005$). However, there were no significant differences in the levels of those with non-complicated disease and those requiring ventilation ($p = 0.115$). When the IL-6 measurements of samples from patients with only COVID-19 were compared (positive PCR), the median IL-6 levels in samples from patients with non-complicated disease ($n = 21$), those requiring ventilation ($n = 47$), and deceased patients ($n = 19$) were 1.14 Log mg/L (IQR: 1.24 Log mg/L), 1.47 Log mg/L (IQR: 0.83 Log mg/L), and 1.89 Log mg/L (IQR: 0.62 Log mg/L), respectively (Figure 3B). IL-6 levels in deceased patients were significantly higher than those in patients with non-complicated disease and those who were intubated ($p = 0.002$ and $p < 0.0001$, respectively), whereas there were no significant differences in the IL-6 levels of those with non-complicated disease and those who required ventilation ($p = 0.331$).

The same comparison analysis was performed with CRP levels between each group. Median CRP levels in samples from patients with non-complicated disease ($n = 20$), those requiring ventilation ($n = 52$), and deceased patients ($n = 17$) were 1.61 Log mg/L (IQR:

FIGURE 2   (A) Comparison between the Cobas and AFIAS assays using Passing-Bablok analysis. The equation of the regression line was y = −0.2781 + 1.068x and the correlation coefficient was 0.967. (B) Difference plot of Cobas and AFIAS measurements using Bland-Altman analysis. Average bias (−0.167 Log pg/ml; SD =0.156 Log pg/ml) is marked by a solid line, and the 95% confidence interval is shown by two dotted lines
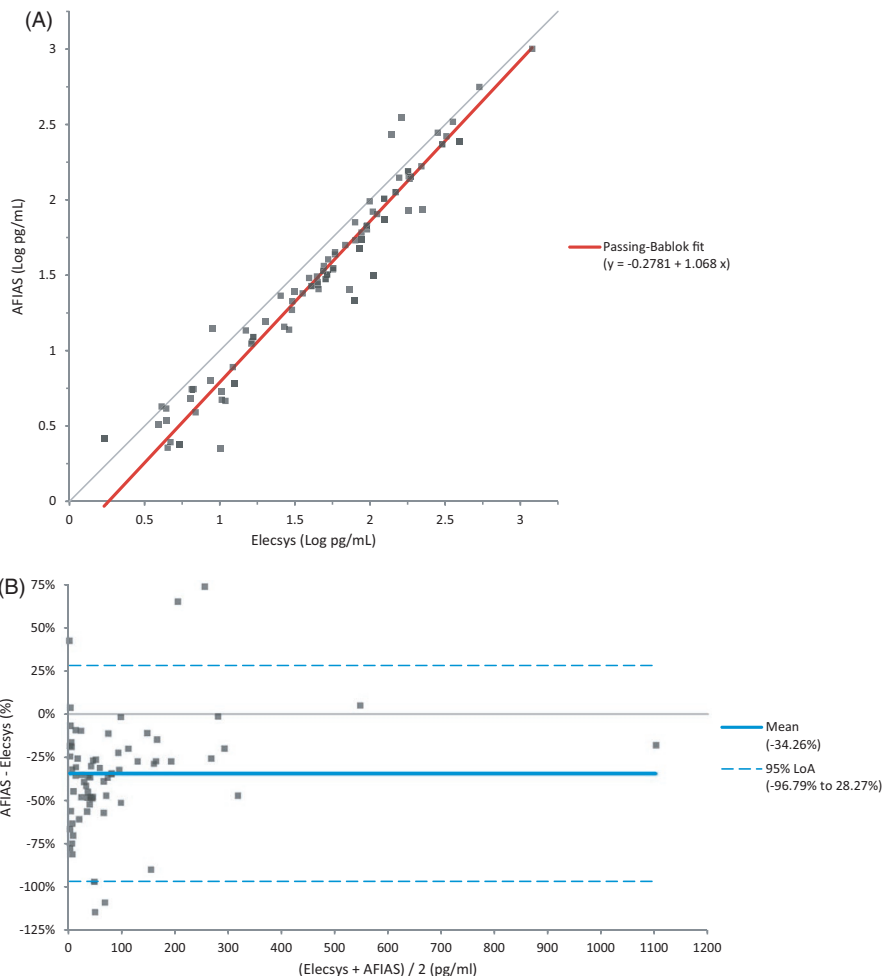

TABLE 4   Classification of IL-6 values measured using the Elecsys and AFIAS assays according to cut-off values stated in the insert paper of each product

IL–6		AFIAS		
		≤7.0 pg/ml	>7.0 pg/ml	Total
Elecsys	≤7.0 pg/ml	22	0	22
	>7.0 pg/ml	8	83	91
	Total	30	83	113

Note: Cohen's coefficient κ: 0.802 (95% confidence interval: 0.736–0.868).

0.57 Log mg/L), 1.74 Log mg/L (IQR: 0.49 Log mg/L), and 1.89 Log mg/L (IQR: 0.58 Log mg/L), respectively. CRP levels in deceased patients were significantly higher than in those with non-complicated disease ($p = 0.024$). However, there were no significant differences in the CRP levels of those with non-complicated disease and those requiring ventilation ($p = 0.092$).

The area under the receiver operating characteristic (AU-ROC) curve (Figure 4) for predicting poor prognosis (deceased patient vs. the rest) was 0.716 (95% CI: 0.594–0.838, $p = 0.006$) for IL-6 and 0.634 (95% CI: 0.488–0.780, $p = 0.086$) for CRP.

4 | DISCUSSION

Overall, we found that the analytical performance of the AFIAS IL-6 assay was comparable to that of the Elecsys IL-6 assay. The assay showed linearity throughout a wide concentration range (2–2,039 pg/ml). The linear range of the assay was wider than that of a previously reported time-resolved lateral flow immunoassay (5–500 pg/ml).[21] The within-run, within-laboratory, between-lot, between-person, and between-site imprecision rates were all <10%, indicating that the assay had good precision. The results yielded by AFIAS IL-6 and Elecsys IL-6 assays showed good agreement (kappa of 0.802). However, significant systemic error was found in the correlation study between AFIAS IL-6 and Elecsys IL-6 assays as the 95% CI of the slope and y-intercept did not include 1.0 and 0.0, respectively. Moreover, the bias percentage was slightly higher (0.66%) than the total error allowable for $p < 0.05$ (33.6%) as defined by Aziz et al.[26] The results obtained by the AFIAS IL-6 assay for whole blood and serum samples exhibited excellent agreement (mean bias =0.024 Log pg/ml; correlation co-efficient =0.971). Furthermore, the comparison analysis between whole blood and serum samples using AFIAS did not yield significant systemic error, although the number of samples was insufficient to reach a robust conclusion. The cut-off value provided
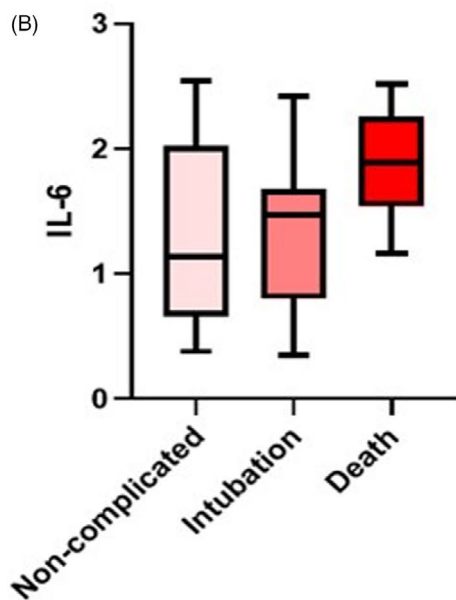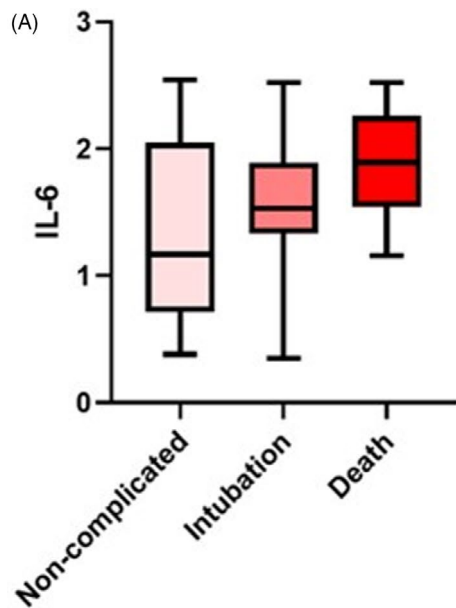
(A)

(B)

FIGURE 3 (A) Box plot of IL-6 levels measured with the AFIAS in relation to disease severity. (B) Box plot of IL-6 levels measured with the AFIAS in relation to disease severity in those with COVID-19

FIGURE 4 ROC curve for predicting patient prognosis (deceased). The area under the receiver operation characteristic (AU-ROC) curve was 0.716 (95% CI: 0.594–0.838, $p = 0.006$) for IL-6 and 0.634 (95% CI: 0.488–0.780, $p = 0.086$) for CRP

for each assay was applied to examine the categorical distribution of the samples used in this study. The two assays exhibited good agreement,[25] and only eight samples showed discrepant results. While the specific values were all near the cut-off value of 7 pg/ml, all eight discrepant samples showed results above the cut-off value on measurement using the Elecsys assay but not with the AFIAS assay.

We found that deceased patients and those who required ventilation had significantly higher IL-6 levels than those with non-complicated disease. An association between elevated IL-6 levels
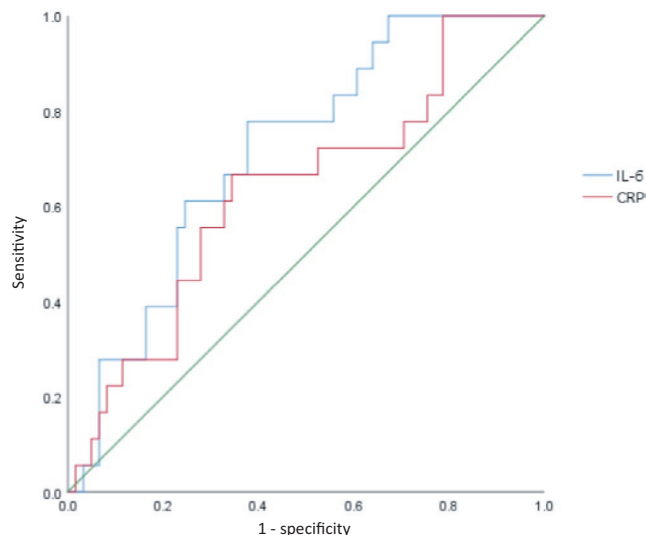
and progression to ARDS and death in COVID-19 patients has previously been observed.[27,28] A meta-analysis of six studies comparing IL-6 levels in patients with complicated vs. non-complicated COVID-19 infections observed 2.9-fold higher levels in complicated COVID-19 cases.[13] The results of our study are consistent with those of previous studies. However, there may be a discrepancy regarding the extent of difference in IL-6 levels, as the clinical condition of the patients may have been tampered due to the inclusion of samples from patients without COVID-19. Specifically, an outpatient clinic patient diagnosed with juvenile idiopathic arthritis had an IL-6 value of 561.49 pg/ml; this may have skewed the distribution of IL-6 values in the non-complicated disease group. Therefore, we conducted the same comparison analysis with samples from patients with only COVID-19. Although there was no change in the final interpretation of this comparison, the differences between the median values of non-complicated and intubated groups vs. the deceased group were greater. In addition to patient diagnosis, another factor potentially affecting this comparison was the exclusion of four samples from the non-complicated disease group, which showed IL-6 values below the LLOD. The fact that we only considered ventilator use instead of ARDS progression may also have mitigated the difference. When the same analysis was performed for CRP levels, significant differences were noted only between patients with non-complicated disease and those who were deceased but not between patients requiring ventilation and those who were deceased. When an ROC curve was drawn, the AU-ROC curve was greater in the case of IL-6 compared to CRP. Therefore, we concluded that IL-6 was a more sensitive predictor of patient prognosis than CRP, and this finding was highly significant within the COVID-19 patient group.

Our study had certain limitations. Firstly, the number of samples analyzed in this study was low. Inclusion of samples from patients with various outcomes (non-complicated disease, ventilator

use, death) may have produced more diverse results. Further, our analysis of IL-6 levels in patients with different outcomes could have yielded a similar degree of difference between the non-complicated and complicated (ventilator use and death) groups, as in previous studies. Additionally, we only considered ventilator use instead of assessing whether each patient showed progression to ARDS.

Despite these limitations, our results demonstrated the overall analytical performance of the AFIAS IL-6 assay was comparable to that of the Elecsys IL-6 assay. Since the AFIAS analyzer does not require strict maintenance, can measure IL-6 levels in whole blood specimens, and involves only a two-step process, no highly trained personnel are required for its processing. Furthermore, the size of the equipment is smaller than those required in conventional methods; thus, it can be used conveniently in the outpatient setting and small laboratories. In addition, the instrument used for the AFIAS IL-6 assay can measure COVID-19 and flu antigens simultaneously. These traits make the AFIAS IL-6 assay an attractive measurement option, especially in light of the ongoing COVID-19 pandemic.

CONFLICT OF INTEREST

The authors declare no competing interests.

AUTHOR CONTRIBUTION

YP and SK contributed to the conception and design. JR and YP contributed to acquisition of data, analysis and interpretation of data, and critical revision of the study for important intellectual content. YJC contributed to drafting the article and statistical analysis., SK and KAL gave administrative support. SK, KAL, and YP are involved in supervision. All authors have accepted responsibility for the entire content of this study and approved its submission.

DATA AVAILABILITY STATEMENT

All authors have access to the entirety of the data underlying this study. Access to the data can be granted at any time upon reasonable request.

ORCID

Younhee Park https://orcid.org/0000-0001-8458-1495

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SUPPORTING INFORMATION

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