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Performance of the Roche IL-6 chemiluminescent immunoassay in patients with COVID-like respiratory symptoms

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ABSTRACT

Introduction: We evaluated the Roche Elecsys IL6 assay on the Cobas immunoassay analyser.
Method: Serum IL6 of 144 controls were compared to 52 samples from patients with COVID-like respiratory symptoms (17 SARS-CoV-2 RT-PCR positive); 25 of these were from the intensive care unit (ICU). We compared the IL6 levels to C-reactive protein (CRP) and procalcitonin (PCT) levels in all cases.

Results: The IL6 assay had coefficient-of-variation (CV) of 2.3 % (34.1 pg/mL) and 2.5 % (222.5 pg/mL), a limit of quantitation < 1.6 pg/mL, and was linear from 1.6 to 4948 pg/mL. There was a significant difference in IL6 values between patients with COVID-like respiratory symptoms versus controls (p < 0.001). ROC analysis showed that IL6 > 6.4 pg/mL identified symptomatic cases (AUC 0.94, sensitivity 88.2 %, specificity 97.2 %). There was a significant difference between the IL6 of symptomatic ICU/non-ICU cases (median IL6 228 vs 11 pg/mL, p < 0.0001); ROC analysis showed IL6 > 75 pg/mL (sensitivity 76.0 %, specificity 88.9 %) was superior to CRP and PCT in predicting ICU admission (AUC: IL6 0.83, CRP 0.71, PCT 0.82).

Conclusion: The performance of Elecsys IL6 assay is in keeping with the manufacturer’s claims. IL6 > 6.4 pg/mL differentiates healthy from suspected COVID-19 cases and appears to be raised earlier than the other inflammatory markers in some cases. IL6 > 75 pg/mL was a good predictor of ICU admission.

1. Introduction

IL6 induces foam cell formation, the release of further inflammatory cytokines, and chemotaxis (Hashizume, 2020). IL6 is elevated in patients with Coronavirus disease 2019 (COVID-19) related cytokine storm syndrome (Huang et al., 2020). Although peak IL6 concentrations in COVID-19 infection are not as raised as in sepsis (Leisman et al., 2020), IL6 can reach up to 430 pg/mL in cases of severe COVID-19 (Herold et al., 2020), and in a study of patients with severe COVID-19 infection requiring intensive care unit (ICU) admission, IL6 was elevated up to 1000-fold compared to healthy controls (Chen et al., 2020). Other studies also support the fact that IL6 is significantly elevated in severe COVID-19 requiring ICU admission (Zhou et al., 2020), and some studies (Valle et al., 2020) have used IL6 to predict survival outcomes in novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. As such, the US Food and Drug administration has recently approved the use of several IL6 assays, for example, the Siemens IL6 assay (Siemens Healthineers, 2021). The Roche Elecsys IL6 assay was approved for use by the US Food and Drug administration in June 2020 (US Food and Drug Administration, 2021) (under emergency use authorization). We describe our evaluation of the Elecsys IL6 assay run on the Cobas e801 immunoassay analyser and compared the IL6 to C-reactive protein (CRP) and procalcitonin (PCT) in subjects with respiratory symptoms suspicious of COVID-19.

Abbreviations: IL6, interleukin-6; SARS-CoV-2, novel severe acute respiratory syndrome coronavirus 2; COVID-19, Coronavirus disease 2019; CRP, C-reactive protein; PCT, procalcitonin; RT-PCR, reverse-transcriptase polymerase chain reaction; HCWs, healthcare workers; LOQ, limit of quantitation; CV, coefficient-of-variation; TCZ, tocilizumab; ICU, intensive care unit.

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2. Methods

2.1. Participants

52 subjects with respiratory symptoms suspicious of COVID-19 (e.g., pneumonia, cough, fever) and tested with RT-PCR from April-June 2020 were recruited as cases (18 females, 34 males), using residual de-identified sera from other routine laboratory testing (e.g., renal panels). 17 of these cases were SARS-CoV-2 RT-PCR positive and 25 cases were from the ICU (9 RT-PCR positive, 16 RT-PCR negative). Samples from 144 (116 females, 28 males) healthy healthcare workers (HCWs) served as controls. As this work involved de-identified leftover sera and was part of evaluating a new diagnostic assay, it was deemed exempt by our institutional review board.

2.2. Methods and materials

The Roche Elecsys IL6 assay is a non-competitive (sandwich) chemiluminescent immunoassay. 18 uL of sample undergoes a first incubation with IL6 specific antibodies, followed by a second incubation with IL6 specific antibodies labelled with ruthenium complexes to form a sandwich complex. Thereafter, complexes are magnetically captured, where a voltage then induces a chemiluminescent emission directly proportional to the IL6 concentration. The assay has a claimed measuring range of 1.5–5000 pg/mL, a limit of quantitation (LOQ) of 2.5 pg/mL, an inter-assay precision (CV) of 17.4 % (at 1.82 pg/mL) and 2.0 % (at 4461 pg/mL). The stated reference interval is <7 pg/mL. For RT-PCR testing, our hospital molecular laboratory employs a duplex real-time RT-PCR that targets the N and E genes using a Qiagen EZ1 extraction system and Rotor Gene Q amplification system. The Elecsys assay is a particle enhanced immunoturbidimetric assay where CRP agglutinates with latex particles coated with monoclonal anti-CRP antibodies; the reference interval for PCT is 0.5 ng/mL.

2.3. Statistical analysis

Statistical analyses were performed using MedCalc® Statistical Software version 19.5.3 (MedCalc Software Ltd, Ostend, Belgium). We compared IL6 values between controls and cases with respiratory symptoms suspicious of COVID-19 (SARS-CoV-2 RT-PCR negative and positive) and between ICU and non-ICU cases. IL6 values were compared between cases and controls using the Mann-Whitney U test, a p-value <0.05 was considered as statistically significant. We also compared the IL6 values between 3 groups: controls, SARS-CoV-2 RT-PCR negative cases with COVID-like respiratory symptoms, and cases positive for SARS-CoV-2 RT-PCR. There was a significant difference (p < 0.0001) between the median IL6 values of the patients with suspected COVID-19 (COVID-like respiratory symptoms with and without positive RT-PCR) and the controls (43.3 vs 2.3 pg/mL), with a difference of 40.4 pg/mL (see Fig. 1a). There was a wide spread of IL6 values between the RT-PCR negative (median 87.2 pg/mL, IQR 7.0–63.5 pg/mL) and RT-PCR positive subjects (median 12.7 pg/mL, IQR 11.1–277.0 pg/mL); the apparent difference between these 2 groups failed to achieve statistical significance (p = 0.052) (see Fig. 1b).

We performed ROC analysis between the IL6 values of the 144 healthy controls and the 52 cases with COVID-like respiratory symptoms. ROC analysis showed that an associated criterion of >6.4 pg/mL could be used to separate cases and controls with a sensitivity of 88.5 % and specificity of 97.2 % (AUC 0.96, p < 0.001) (see Fig. 2). We also compared the IL6, CRP and PCT values between ICU cases and non-ICU cases. There was a significant difference between the IL6 values of both groups (median IL6 228 vs 11 pg/mL, p < 0.0001) (see Fig. 3). Similarly, CRP and PCT was also significantly different in these groups (CRP 338 vs 210 mg/L, p = 0.009; PCT 1.49 vs 0.13 ng/mL, p = 0.0001 respectively).

We also performed ROC analysis for admission to the ICU in our patients. IL6 levels predicted ICU admission with an AUC of 0.83 (95 % CI 0.70 to 0.92, p < 0.001) at an associated criterion of >75 pg/mL (sensitivity 76.0 %, specificity 88.9 %) (see Fig. 4). The predictive value of IL6 was superior to that of CRP (AUC 0.71, 95 % CI 0.57 to 0.83) and PCT (AUC 0.82, 95 % CI 0.69 to 0.91).

3. Results

3.1. Performance analysis

The Elecsys IL6 assay had a CV of 2.3 % (34.1 pg/mL)/2.5 % (222.5 pg/mL). The assay was linear from 1.6 to 4948 pg/mL. The LOQ was deemed to be 1.6 pg/mL, as CV at this level was still 4.9 % (95 % CI 2.5–7.3 %). This is lower than the manufacturer stated LOQ of 2.5 pg/mL. Assay time was 18 min and results were available 1 min later; throughput for the analysis of 50 samples was 29 min. For computation of results, values <1.6 pg/mL are taken as 1.6 pg/mL, and values reported as >5000 pg/mL are taken as 5000 pg/mL.

3.2. Comparison of IL6 values between groups

We compared the IL6 values between 3 groups: controls, SARS-CoV-2 RT-PCR negative cases with COVID-like respiratory symptoms, and cases positive for SARS-CoV-2 RT-PCR. There was a significant difference (p < 0.0001) between the median IL6 values of the patients with suspected COVID-19 (COVID-like respiratory symptoms with and without positive RT-PCR) and the controls (43.3 vs 2.3 pg/mL), with a difference of 40.4 pg/mL (see Fig. 1a). There was a wide spread of IL6 values between the RT-PCR negative (median 87.2 pg/mL, IQR 7.0–63.5 pg/mL) and RT-PCR positive subjects (median 12.7 pg/mL, IQR 11.1–277.0 pg/mL); the apparent difference between these 2 groups failed to achieve statistical significance (p = 0.052) (see Fig. 1b).

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3.3. Comparison with CRP and PCT

Studies (Leisman et al., 2020) have shown that the estimated pooled mean IL6 in COVID-19 was 37 pg/mL. Based on this cut-off, we stratified the IL6 values in our population. When cases were grouped based on initial IL6 levels (<6.4 pg/mL, 6.4–37 pg/mL, >37 pg/mL), CRP and PCT generally increased in tandem with IL6 (see Table 1). 8 out of 52 patients had a normal normal CRP and PCT; IL6 was >6.4 pg/mL in 5 of them. Two of them had IL6 of 5000 pg/mL and 2088 pg/mL. CRP (5.9 pg/mL, 2.9 mg/mL, PCT 0.05 and 0.25 ng/mL respectively). Notably, all 6 cases in our study with IL6 >1000 pg/mL (2088–5000 pg/mL) were all ICU cases but were SARS-CoV-2 RT-PCR negative.

4. Discussion

The Elecsys IL6 assay has good precision (<2.5 %), linearity (1.6–4948 pg/mL), LOQ (<1.6 pg/mL) and throughput (50 samples in 29 min). There is a significant difference between the IL6 values of healthy patients and those with respiratory symptoms suspicious for COVID-19, and our IL-6 cut-off value >6.4 pg/mL from ROC analysis is close to the manufacturer’s stated cut-off of >7.0 pg/mL. In other studies that compared the IL6 levels, between 465 COVID-19 survivors and 36 non-survivors (Laguna-Goya et al., 2020), there was a significant difference between the IL6 levels (median 17.0 pg/mL vs 80.0 pg/mL). In a meta-analysis of 11 studies with 1357 patients (Motahhari et al., 2020), IL6 was higher in patients with critical COVID-19 compared to mild disease (mean difference of 23.1 pg/mL). However, we found no significant difference in IL6 levels between SARS-CoV-2 RT-PCR positive and negative cases with COVID-like respiratory symptoms. Part of the
issue is how great variation exists between the performance of RT-PCR assays (Igloi et al., 2020). When the College of American Pathologists surveyed more than 700 laboratories with control materials, the median cycle threshold values reported for different methods varied by as much as 14 cycles (Rhoads et al., 2020), and even within the same instrument, the difference in median cycle threshold values for different targets was as high as 3.0 cycles. This may have contributed to the lack of distinction in IL6 values between RT-PCR negative and positive cases in our study.

Secondly, IL6 is not specific to COVID-19 and can be raised in any cause of ARDS and sepsis (Leisman et al., 2020) and in many disorders with chronic inflammation (e.g., rheumatoid arthritis) (Tanaka et al., 2014). In addition, our COVID-19 caseload in this study was modest (n = 17).

Despite this, IL6 still has use in patients suspicious for COVID-19. As shown in our study, the elevation of IL6 precedes that of CRP and PCT in some of our patients (9.6 %, 5 out of 52 cases). Of note, two of these ICU cases had extremely high IL6 levels (>2000 pg/mL) compared to their corresponding low CRP/PCT. In another study of 140 patients with COVID-19 (Liu et al., 2020), 67.9 % (95/140) had a raised IL6 on
admission, whereas only 65.0 % (91/140) had a raised CRP and 5.7 % (8/140) had a raised PCT. More evidence of the early rise in IL6 compared to CRP and PCT has been reported in earlier studies, for example, in a study of mortality in 328 patients with severe sepsis and septic shock (Weidhase et al., 2019). IL6 values 48–72 hours after admission was significantly lower in survivors than non-survivors (114.2 vs 746.6 pg/mL); however, PCT (5.6 vs 4.9 ng/mL, p = 0.586) and CRP (158.5 vs 172.4 mg/L, p = 0.988) values showed no significant difference. Furthermore, IL6 had a higher AUC than PCT and CRP at 24 h (IL-6: 0.701, PCT: 0.594, CRP: 0.490) and 48–72 hours (IL-6: 0.792, PCT: 0.650, CRP: 0.584) after admission. Another study that compared IL6 to PCT in sepsis and septic shock (Song et al., 2019) showed that IL6 was better able to discriminate between sepsis and controls (AUC 0.89) while the AUC of CRP and PCT were 0.77 and 0.80 within 6 h of the diagnosis of sepsis. The cumulative evidence suggests that IL6 is an earlier biomarker of severe infections than CRP or PCT.

IL6 of >75 pg/mL was also superior to CRP and PCT in the prediction of ICU admission, and there was a significant difference in IL6 between ICU and non-ICU cases. This is supported by other studies as well. In one study (Herold et al., 2020), maximal IL6 levels before intubation was the best predictor for mechanical ventilation, with an AUC of 0.97 compared to an AUC of 0.86 for CRP; presentation IL6 levels >35 pg/mL had a high sensitivity (84 %) for respiratory failure. The same study (Herold et al., 2020) also showed that IL6 levels could predict respiratory failure earlier than CRP (23.2 vs 15.7 h). In another study of 901 COVID-19 cases (Zhang et al., 2020), an IL6 concentration >38 pg/mL was predictive of mortality with an AUC of 0.97, with critical cases of COVID-19 having a higher baseline IL6 compared to milder infections. A meta-analysis (Aziz et al., 2020) comparing IL6 in severe and non-severe COVID-19 patients (n = 1426) showed a clear distinction between IL6 in these 2 states (mean IL6 56.8 vs 17.3 pg/mL, p < 0.001). Using a strict definition for respiratory distress, admission IL6 > 80 pg/mL had a clear association with mortality. In other studies (Luo et al., 2020; Galvan-Roman et al., 2021), IL6 levels as low as 20–30 pg/mL were accurate prognostic markers for COVID-19 mortality or invasive mechanical ventilation. The magnitude of IL6 increase can thus aid clinicians in their decision to consider more intensive care for patients with COVID-like respiratory symptoms.

Our study presents the following novel findings:

- We confirm that an IL6 level of >6.4 pg/mL can be used to differentiate symptomatic from healthy subjects
- In symptomatic cases, there was no significant difference between the IL6 levels of SARS-CoV-2 RT-PCR positive and negative cases.
- There is a significant difference between the IL6 levels of ICU cases and non-ICU cases.
- In cases with respiratory symptoms suspicious of COVID-19, an IL6 of >75 pg/mL can be used to predict ICU admission.
- IL6 levels are raised earlier than CRP and PCT in some symptomatic cases.

A limitation of our study is the small sample size (n = 52) and few (n = 17) SARS-CoV-2 RT-PCR positive cases. Besides, information on the clinical diagnosis, inpatient history, disease severity and medication record of all subjects was not available. We also had no information on the CRP and PCT in the control group and were thus unable to compare the CRP and PCT between cases and controls. Although IL6 was originally considered a monitoring tool in patients treated with Tocilizumab (TCZ) (IL6-blockade) in COVID-19, its clinical use has waned. In the latest phase III COVACTA trial (Roche Diagnostics, 2021) in COVID-19 pneumonia, there was no statistically significant difference between patients on TCZ and those on a placebo; with no difference in the improvement of clinical status (odds ratio 1.19, 95 % CI 0.81–1.76, p = 0.36) or mortality (19.4 % mortality in both groups). Another study (Stone et al., 2020) showed that TCZ was not effective for averting intubation or death in COVID-19 patients with impending respiratory failure (HR 0.83 compared to controls). In addition, in a study of 129 patients with COVID-19 who were receiving supplemental oxygen or mechanical ventilation (Veiga et al., 2021), 17 % of patients in the TCZ group died compared to only 3 % in the standard care group, with 43 % of patients in the tocilizumab group having adverse events compared to 34 % of the control group. On the other hand, in a study of 764 patients requiring ICU support, an association was noted between receiving TCZ and decreased mortality (HR 0.64) (Biran et al., 2020). In yet another study of 64 patients, the use of TCZ in earlier stages of disease resulted in an early favourable response in 76.6 % of cases (Guillen et al., 2020). Of note, the IL6 patterns were different between responders and non-responders to TCZ, with a recommended cut-off value of 177 pg/mL to differentiate between the two groups. A recent study comparing TCZ treatment against a placebo in 389 COVID-19 patients (Salama et al., 2020) showed that although TCZ reduced the likelihood of disease progression (12 % with TCZ vs 19 % without TCZ), it did not improve survival (mortality 11.6 % with TCZ and 11.8 % without TCZ). The conflicting evidence of IL6-blockade in COVID-19 treatment has led to declining enthusiasm for its use as a treatment regimen and hence utilization of IL6. Indeed, in the latest guideline by the US National Institutes of Health (2021), the panel had no recommendation for or against the use of TCZ in the treatment of COVID-19 for patients who were within 24 h of admission to the ICU requiring respiratory support. Although we did not have access to the medication history of the subjects in this study, TCZ use for COVID-19 treatment is approved for clinical trials only in our country, and our centre was not a study centre for TCZ treatment. We understand that none of the symptomatic cases in our study received TCZ treatment.

5. Conclusion

The Roche Elecsys IL6 assay performs as claimed by the manufacturer but we found that the LoQ is lower. There is a clear difference between the IL6 of cases with respiratory symptoms suspicious of COVID-19 and healthy controls. IL6 may rise earlier than CRP and PCT in some cases with COVID-like respiratory symptoms, and IL6 is a good predictor for ICU admission in symptomatic cases.

Disclosures

All co-authors have contributed to the study and manuscript.

Declaration of Competing Interest

None.

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