# A Point-of-Care Electrochemical Biosensor for Early Detection & Monitoring of Periprosthetic Joint Infection

Puranjay Gupta[1], Vaishnavi Guruprasad[1], Mareeswari Paramasivan[1], Edith Zhan[1], John Hamilton[2], Markus A Wimmer[2], Mathew T. Mathew[1]
*[1]Regenerative Medicine and Disability Research Lab, University of Illinois College of Medicine Rockford, Rockford, IL, [2]Rush Orthopedics, Chicago, IL*
*Email: puranjay@uic.edu; mtmathew@uic.edu*

**Disclosures:** Puranjay Gupta (N), Vaishnavi Guruprasad (N), Mareeswari Paramasivan (N), Edith Zhan (N), John Hamilton (N), Markus A Wimmer (N), Mathew T. Mathew (N)

**INTRODUCTION:** Periprosthetic joint infection (PJI) is a leading cause of implant failure, responsible for 21.4% of hip and 33.1% of knee implant revisions.[1] These corrective surgeries are costly, exceeding $100,000 per case, and have a high reinfection rate of nearly 42%.[2,3] Standard diagnostics, such as arthrocentesis for synovial fluid culture followed by imaging, are often performed late in the disease progression, by which point significant tissue damage may have occurred. While other detection methods like ELISA and PCR exist, they are time-consuming, expensive, require specialized personnel, and are only used when symptoms from PJI manifest. Earlier detection of PJI could increase the chances of bacterial eradication, minimize patient pain, and reduce the likelihood of implant failure by allowing timely interventions such as targeted antibiotic therapy or debridement procedures. Electrochemical biosensors have emerged as promising tools for rapid, point-of-care detection of disease biomarkers in various fields. Established biomarkers for PJI include local and systemic inflammatory markers such as interleukin-6 protein 6 (IL6) and C-reactive protein (CRP), which have reported sensitivities for PJI diagnosis between 80-90%.[4] Therefore, we aim to develop a portable, sensitive, and inexpensive electrochemical biosensor for the early detection of PJI. We hypothesize that the biosensor, coupled with a machine learning (ML) based classifier, will quantify these biomarkers in blood serum and use these patterns to detect and monitor PJI.

**METHODS: 1) Biosensor Modeling:** A three-electrode system was utilized, consisting of a gold working electrode modified with graphene oxide to increase the electroactive surface area and sensitivity, a counter electrode, and a reference electrode. To determine the optimal antibody loading, anti-IL6 and anti-CRP antibodies were immobilized on the working electrode using a dithiobis(succinimidyl propionate) (DSP) crosslinker at different concentrations (0.25, 0.5, 1.0, 1.25, and 1.5 µg/mL). The sensor's performance was evaluated using electrochemical impedance spectroscopy (EIS) to obtain change in impedance ($\Delta Z$), capacitance ($C_P$), resistance ($R_P$), and cyclic voltammetry (µC). Following optimal antibody loading, a protein detection curve was established using IL6 concentrations representative of different clinical risk strata derived from literature analyzing patient serum with PJI: normal (2-5 pg/mL), low risk (5–20 pg/mL), and high risk (20+ pg/mL).[5] A CRP calibration curve was established from similar literature: normal (<3 µg/mL), low risk (3-10 µg/mL), and high risk (10+ µg/mL).[6] Antibody-antigen binding interactions were confirmed using confocal microscopy with FITC-secondary antibody stain. **2) Biological Validation:** To validate the biosensor's classification of PJI, a simulated infectious environment was created from lipopolysaccharide (LPS)-treated human fibroblast-like synoviocytes (HFLS) cells (10, 100, 1000 ng/mL of LPS at 24, 48, and 72h). Blinded serum tests were also conducted with unknown concentrations of IL6 and CRP. ELISA and confocal microscopy were used to estimate established concentrations of IL6 and CRP in the supernatant and blinded samples. The samples were then classified using the biosensor model and compared to the estimated values. **3) Machine Learning Model:** The collected data were used to train a support vector ML model and a neural network to classify and predict PJI based on protein concentration levels.

**RESULTS: 1) Biosensor Modeling:** The optimal anti-IL6 antibody and anti-CRP antibody concentration for immobilization was determined to be 1.0 µg/mL and 0.5 µg/mL, respectively. EIS analysis demonstrated a clear, dose-dependent increase in impedance ($\Delta Z$), capacitance ($C_P$), charge transfer resistance ($R_P$), and cyclic voltammetry (µC), upon exposure to increasing IL6 concentrations, confirming effective detection across the entire clinically relevant range **(Figure 1a)**. Similar trends were noted from the CRP protein calibration **(Figure 1b)**. Confocal microscopy confirmed successful antibody immobilization and specific IL6 and CRP binding. **2) Biological Validation:** Confocal microscopy confirmed the presence of IL6 in the LPS-treated cell supernatant and blinded samples **(Figure 1c-d)**. Fluorescence intensity analysis using standard curves classified IL6 and CRP concentrations as high risk in the *in vitro* models, and high and low risk in the blinded samples, respectively. ELISA confirmed protein concentrations were consistent with risk levels, specifically estimating IL6 at >20 pg/mL (high risk) and CRP at approximately 5 µg/mL (low risk) in the blinded samples **(Figure 1e-f)**. The biosensor accurately classified the LPS-treated model and the blinded samples. **3) Machine Learning Model:** Following model training, validation data confirmed successful classification of PJI with IL6 and CRP at 70.77% and 72.41% respectively **(Figure 1g-h)**.

**DISCUSSION:** In the current study, electrochemical biosensors have been initially validated as a potential tool for the early detection of PJI. The sensors successfully classified simulated infectious supernatant and blinded samples relative to ELISA and fluorescent intensity, based on IL6 and CRP concentrations. IL6 and CRP were selected for this preliminary study as they are well-characterized cytokines and are strongly recommended by the American Association of Orthopaedic Surgeons as diagnostic markers for PJI. As demonstrated, the lab-designed biosensor can detect concentrations of IL6 and CRP in simulated infectious conditions. Notably, the sensor detected IL6 in the low picogram range and CRP in the low microgram range, surpassing detection thresholds required for early-stage diagnosis. Furthermore, support-vector ML models demonstrated an initial successful classification of PJI from IL6 and CRP individually, providing a proof of concept that complements the higher reported sensitivities of these biomarkers in the literature. Integrating these datasets will enhance the specificity of the biosensor tests. While IL6 and CRP are strong indicators of PJI, it is recognized that two biomarkers are insufficient for a definitive PJI diagnosis. Future work will integrate additional PJI biomarkers, such as D-dimer, to develop an electrochemical sensor panel that enhances the diagnostic accuracy of our ML models for the early and reliable prediction of PJI. Despite these limitations, this integration of electrochemical biosensors and ML represents a key step toward developing intelligent diagnostic tools that could be deployed in outpatient settings.

**CLINICAL RELEVANCE:** Periprosthetic joint infection is a devastating complication of joint replacement with high morbidity, financial burden, and reinfection risk. While current diagnostics are delayed and invasive, this study presents a novel, point-of-care electrochemical biosensor capable of detecting IL6 and CRP with high sensitivity in clinically relevant concentrations to diagnose PJI early.

**REFERENCES:** [1] AJRR *et al*., 2025 [2] Patel *et al*., 2023. [3] Gold *et al*., 2019 [4] Li *et al.,* 2020 [5] Xie *et al*., 2017 [6] Alijanipour *et al*., 2013.

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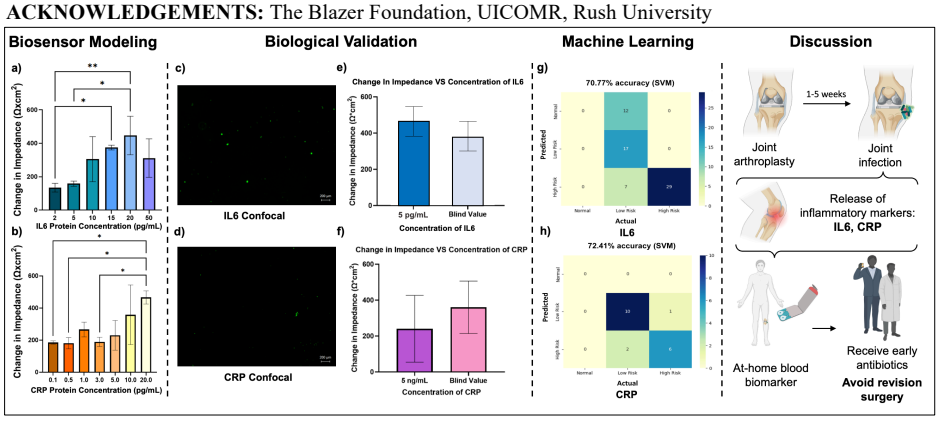


**Figure 1: Biosensor Modeling.** Protein calibration curves were created from a range of clinically relevant concentrations. **a)** IL6 **b)** CRP. **Biological Validation.** Confocal microscopy confirmed the presence of **c)** IL6 and **d)** CRP in blinded samples. Biosensor models accurately classified **e)** IL6 and **f)** CRP concentrations based on the change in impedance. **Machine Learning.** Support-vector model achieved **g)** 70.77% accuracy with IL6 and **h)** 72.41% accuracy with CRP. **Discussion.** Mechanism for biosensor function and clinical implementation.