



# Are C-reactive protein (CRP) and neutrophil to lymphocyte ratio (NLR) predictive markers of successful two-stage prosthetic joint infection management?

M. ECKI<sup>1</sup>, H. POILVACHE<sup>1-3</sup>, M. RANDY BUZISA<sup>1-3</sup>, M. VAN CAUTER<sup>1-3</sup>, H. RODRIGUEZ-VILLALOBOS<sup>2</sup>, J.-C. YOMBI<sup>1</sup>, O. CORNU<sup>1-3</sup>

<sup>1</sup>Orthopaedic and Trauma Department, Cliniques universitaires Saint-Luc UCL, 1200 Brussels, Belgium; <sup>2</sup>Microbiology Department, Cliniques universitaires Saint-Luc UCL, 1200, Brussels, Belgium; <sup>3</sup>NMSK, IREC, Université catholique de Louvain, 1200 Brussels, Belgium.

Correspondence at: Martin Ecki, 18 Avenue Paule; BE-1310 La Hulpe, Belgium, Phone: +32 (0)479 54 91 90, Email: martin.ecki@student.uclouvain.be

**The treatment of prosthetic joint infections (PJI) regularly involves a two-stage surgical strategy. The second stage is generally performed when the surgeon estimates that the infection is under control based on either the treatment length or clinical and biological data. We have raised the question whether standardization of C-reactive protein (CRP) and neutrophil to lymphocyte ratio (NLR) values could be either indicative of infection control or predictive of infection recurrence.**

**This was an analytical retrospective and monocentric cross-sectional observational study. The CRP and NLR values were recorded prior to the second-stage surgery in a sample of 100 patients who underwent a two-stage revision arthroplasty following a PJI, and these values were correlated with the absence of infectious recurrence within a 2-year follow-up. The statistical analysis consisted of evaluating the diagnostic validity of each marker, first individually, and then in combination.**

**CRP was shown associated with a 68% sensitivity, 40% specificity, 27% positive predictive value (PPV), and 79% negative predictive value (NPV). The ROC curve was 51.1%. The NLR displayed a 12% sensitivity, 89% specificity, 27% PPV, and 75% NPV. The ROC curve was 47.9%. The combination of the two markers did not fundamentally improve the statistical results, with only a 43% concordance of the two markers, 27% sensitivity, 84% specificity, 37% PPV, and 77% NPV.**

**Measuring CRP and NLR values, either individually or in combination, before the second-stage revision arthroplasty did not turn out to be predictive of either infection recurrence or cure within two years of follow-up. Therefore, an absolute test is still lacking, which would enable us to predict without failure the absence of control or the control of a PJI before or after second-stage revision.**

*Level of evidence:* Level III, retrospective cross-sectional study.

## INTRODUCTION

Numerous studies<sup>1,2</sup> have been focused on prosthetic joint infections (PJI), primarily concerning diagnostic methods for this pathological condition. However, a more limited number of publications<sup>3</sup> have so far concentrated on the methods to confirm either a PJI's cure or its recurrence. Given that the treatment of PJIs generally consists of a revision of the implant, it is crucial for the surgeon to better understand the probability of infection recurrence before carrying out a procedure revision and implementing a new prosthesis. Due to the lack of serological markers that allow for PJI diagnosis, we wished to understand whether there were serological markers able to predict a PJI recurrence.

This study sought to evaluate the ability of C-reactive protein (CRP), neutrophil-lymphocyte ratio (NLR), as well as their association to predict a PJI recurrence before the second-stage revision procedure. To this end, we performed a diagnostic validity test of CRP and NLR based on a retrospective cross-sectional study. The sample on which this study was conducted consisted of a group of patients who underwent a two-stage revision of their hip or knee prosthesis on account of PJI.

## MATERIALS AND METHODS

This study was an analytical observational, retrospective, cross-sectional, and monocentric study, which

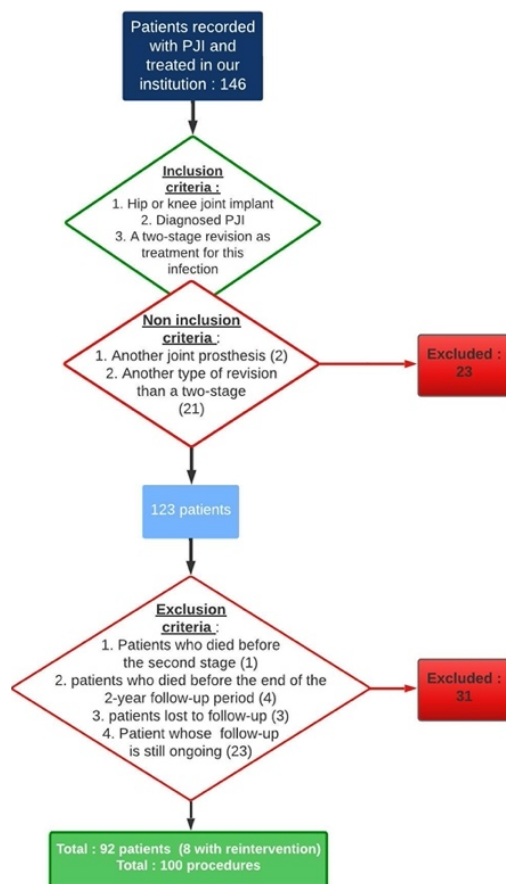


Figure 1. — Flowchart of patient selection.

was authorized by the Ethics Committee (Reference B403201523492). We reviewed the medical records of the patients admitted to the *Cliniques universitaires Saint-Luc* in Brussels for a PJI, with 92 patients (for a total of 100 interventions) eventually retained from 146 patients initially considered (Figure 1).

The inclusion criteria were as follows: 1) having a hip or knee joint implant; 2) PJI diagnosis according to MSIS criteria; 3) having undergone a two-stage revision prosthesis for managing this infection. The non-inclusion criteria were as follows: 1) having another joint implant (n=2 patients); 2) having undergone another revision type than a two-stage revision (n=21). Finally, the exclusion criteria were as follows: 1) Patients who died before second-stage surgery (n=2), patients who died before the end of the 2-year follow-up (n=4), patients lost to follow-up (n=3), patients with no two-year follow-up data, with follow-up still ongoing (n=23) at study end.

CRP is a biological marker that has already proven its value in the diagnosis of infectious diseases<sup>4</sup>. The normalization of this marker is an essential sign during postoperative arthroplasty follow-up. In our study, the reference value used considered CRP to be normal if <1mg/dL. The NLR was obtained from the white

blood cell count (WBC), corresponding to the ratio of neutrophil to the lymphocyte counts. This marker is reflective of the balance between two aspects of the immune system, meaning inflammation and adaptive immunity. It has primarily been studied in the cardiology and oncology domains, but also in orthopedics<sup>5</sup>, where it has been proven to be a prognostic factor for both mortality and cardiovascular complications. More recently, a study has attempted to highlight NLR's ability to predict prosthesis infection<sup>6</sup>. In our study, NLR <5 was considered to be the reference normal value.

These two markers were measured before second-stage surgical revision, within maximally seven days before the intervention. We respected a 6-week period between the two-stage revision in order to allow for antibiotics to be administered, clinical and biological evolution of patients to be followed-up, and the infection be better controlled before prosthesis reimplantation<sup>7</sup>.

A diagnostic validity test was carried out for each of the markers to establish its possible diagnostic power for predicting PJI recurrence. To this end, we compared our two tests with a reference diagnostic test considered as “Gold standard”. In our study, the Gold standard consisted of the patient follow-up at two years after the revision arthroplasty. Thus, patients were qualified as cured of their infectious episode if they presented no clinical, biological, and radiological signs in favor of an infection within two years of second-stage surgical revision. The diagnostic validity study of both CRP and NLR enabled us to highlight different validity criteria for each marker, as follows: 1) Intrinsic validity consisting of sensitivity and specificity from which it is possible to determine the ratio of the test's likelihood, as well as the ROC curve; 2) extrinsic validity consisting of positive and negative predictive values that were essential to know whether the tests studied were predictive of infection recurrence.

We additionally considered other variables to better understand whether they influenced the risk of infection recurrence. These variables that possibly lead to a bias upon analyzing the link between the variable studied and pathological condition considered were: gender, patient age, infection type, germ, and number of comorbidities. For the analysis of these factors and their influence, a correlation matrix along with a multivariate analysis was employed.

## RESULTS

The CRP results concerning the prediction of infection recurrence at two years were inconclusive (Table 1).

**Table 1.** — Contingency table and CRP results

	Relapse after T2	No relapse after T2	Total :
CRP > 1	17	45	62
CRP < 1	8	30	38
Total :	25	75	100

CRP	
Sensibility	68% [CI : 48,41% to 82,79%]
Specificity	40% [CI : 29,66% to 51,31%]
LH+	1,13 [CI : 0,818 to 1,5711]
LH -	0,8 [CI : 0,424 à 1,51]
Youden Index	0,08
Diagnostic efficiency	47%
ROC Curve :	
- Best threshold :	1,35 (Se 60%, Sp 48%)
- AUC :	51,1% [CI : 38,5% à 63,8% ]
PPV	27,42% [CI : 16,3% à 38,5% ]
NPV	78,95% [CI : 66% à 91,9% ]
Chi-Square	0,51
Diagnostic gain	2,42%

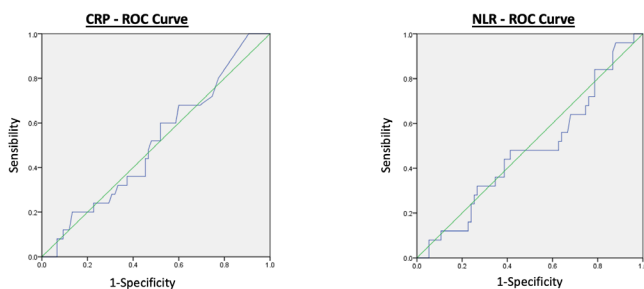
Low specificity results due to the high number of false positives (45/75 = 60%): 60% of patients with a positive test did not actually develop a relapse at two years. The NLR is close to 1, meaning that a patient who will develop an infection recurrence within the two years following the revision has almost as much probability of having a positive as a negative CRP at the second stage. Measuring CRP is therefore not an informative test. The Chi-squared value obtained does not enable us to confirm that there is a statistically significant link between CRP and prosthesis infection recurrence. CRP: C-reactive protein; NLR: neutrophil to lymphocyte ratio.

**Table 2.** – Contingency table and NLR results

	Relapse after T2	No relapse after T2	Total :
NLR > 5	3	8	11
NLR < 1	22	67	89
Total :	25	75	100

NLR	
Sensibility	12% [CI : 4,17% to 29,96%]
Specificity	89,33% [CI:80,34% to 94,50%]
LH+	1,12 [CI : 0,323 to 3,916]
LH -	0,98 [CI : 0,278 à 4,685]
Youden Index	0,01
Diagnostic efficiency	70%
ROC Curve :	
- Best threshold :	2,71 (Se 48%, Sp 58,7%)
- AUC :	47,9% [CI : 34,9% to 61,0% ]
PPV	27,27% [CI : 1% to 53,6% ]
NPV	75,28% [CI : 66,3% to 84,2% ]
Chi-Square	0,03
Diagnostic gain	2,27%

The NLR measurement is much less sensitive than that of CRP, but on the other hand, it exhibits a much greater specificity. There is only a 27.27% chance that a patient with high CRP values before the second-stage will actually experience an infection relapse within the following two years. The Chi-squared value obtained does not enable us to confirm the existence of a statistically significant link between measuring NLR and infection recurrence within two years. CRP: C-reactive protein; NLR: neutrophil to lymphocyte ratio.



*Figure 2.* — ROC curves of CRP and NLR.

By performing the ROC curve (Figure 2), the AUC was 51.1%, meaning that CRP represented a poor test to discriminate patients who were likely to relapse from those who were not. In addition, the Chi-squared value obtained in our study (0.51) did not enable us to confirm that a statistically significant link existed between CRP and PJI recurrence. Finally, we can now state that measuring CRP before second-stage is not an efficient test to predict infection recurrence within the

two years of a revision arthroplasty procedure, as based on the results developed above, with a proportion of correct results of only 47%, Youden index of 0.08, and diagnostic gain of 2.42%.

The results obtained for the NLR were likewise inconclusive (Table 2). By performing the ROC curve (Figure 2), the AUC obtained (47.9%) was even lower than CRP's one. The NLR had thus no power to discriminate patients who were likely to relapse from those who were not. The Chi-squared value, which was even lower than that of CRP, did neither define the existence of a statistically significant link between the marker and infection recurrence at two years of the revision. Therefore, we can finally admit that measuring NLR before second-stage is not an efficient test to predict infection recurrence within two years of a revision arthroplasty procedure given the results presented above, a Youden index, and diagnostic gain even lower than those obtained for CRP.

Testing the CRP and NLR in combination did not fundamentally change the statistical results (Table 3), which remained weak. First, the two tests only showed a concordance of 43%, meaning that in more than half

**Table 3.** – Contingency table and results for combining CRP-NLR

	Relapse after T2	No relapse after T2	Total :
2 tests +	3	5	8
2 tests -	8	27	35
Total :	11	32	43

	Combinaison CRP-NLR
Sensibility	27,27% [CI:9,75% to 56,56%]
Specificity	84,38% [CI : 68,25 to 93,14]
LH+	1,74 [CI : 0,583 to 6,134]
LH –	0,86 [0,583 to 1,275]
Youden Index	0,12
Diagnostic efficiency	69,77%
PPV	37,50% [CI : 4% to 71% ]
NPV	77,14% [CI : 21,5% to 53,5% ]
Chi-Square	0,73
Diagnostic gain	12,50%

The specificity of the CRP-NLR combination (84.38%) is higher than that of CRP and NLR taken individually. The positive predictive value is greater for the CRP-NLR couple with a value of 37.50%. The CRP-NLR combination offers the best diagnostic gain in our study (12.50%).

of the cases, the CRP and NLR did not vary in the same way depending on the patient infectious states. However, for concordant cases, the combination of these two tests did offer better results than either the CRP or NLR test performed alone. Similarly, the positive likelihood ratio was greater for the combination than for each test taken separately. There was a 1.8 times greater chance of having a positive CRP and NLR couple in a patient who would develop an infection recurrence within the two years of second-stage. In our analysis, while the CRP-NLR combination offered the highest diagnostic gain, the Chi-squared test turned out to be too low to confirm a statistically significant link between measuring this combination and infection recurrence. Therefore, there was a slight advantage in measuring CRP and NLR in combination, because the diagnostic probabilities were better in the event these two tests turned out to be concordant. Nevertheless, as these results were rather weak, this test combination cannot be considered a good diagnostic test to predict PJI recurrence within two years of a revision arthroplasty procedure.

Neither the correlation matrix nor the multiple logistic regression revealed a statistically significant association between these variables and PJI recurrence within two years post-revision. We thus concluded that the patient’s gender, age, infection type, pathological germ, and number of comorbidities present are not considered determinants of the patient’s infection state of the second stage revision procedure.

## DISCUSSION

The CRP results were not surprising. Indeed, several publications studied the role of CRP prior to performing second-stage revision-arthroplasty in the PJI setting, with reported results similar to ours. The first publication<sup>8</sup> sought to determine CRP’s prognostic value prior to performing second-stage revision of infected knee prostheses, again with reported results almost identical to ours. The second publication<sup>9</sup> looked at CRP’s ability to predict recurrent infection. In this study, only one out of eight patients who did not normalize their CRP values before second-stage revision actually exhibited infection recurrence. Moreover, the seven patients who actually displayed infection recurrence following revision, all presented values within the normal range. Based on these data, the authors concluded that this serological marker was rather a poor tool for predicting infection recurrence following revision procedure. These disappointing results could be partly explained by CRP kinetics. Indeed, the CRP plasma levels were shown to fluctuate after an arthroplasty procedure<sup>10</sup>, with the maximum values classically obtained on second or third post-intervention day, then gradually decreasing so as to normalize after three to six weeks. That being said, should CRP values be still abnormal at six weeks postoperatively, this does not necessarily point towards the presence of a prosthesis infection, as highlighted in another publication<sup>11</sup>. Therefore, a pathological CRP value recorded before second-stage does not necessarily indicate infection persistence, given that this second-stage is usually performed around 4 to 6 weeks following the first stage.

Unlike the CRP results, the NLR results collected in our study cannot be accounted for by the NLR kinetics. Indeed, a study conducted in 2015<sup>12</sup> compared the CRP and NLR kinetics by measuring them at different times post-intervention. The results demonstrated that NLR normalized faster than CRP. According to the authors, on Day 21 post-intervention, only 4.5% of patients presented with abnormal NLR values, whereas 20% of them still displayed an abnormal CRP. Therefore, NLR’s kinetic profile very likely differs from that of CRP, as it normalizes earlier and returns to normal values more quickly than does that of CRP. Nevertheless, our results could, at least to some extent, be explained by our choice of a threshold value of 5, the latter being most likely too high. Indeed, a study<sup>13</sup> attempted to demonstrate NLR’ ability to predict PJI diagnosis. Using a ROC curve, the authors were able to determine the optimal NLR threshold value able to discriminate

infected from uninfected patients. This cut-off value, which was 2.45, was associated with a 90% sensitivity % and 72% specificity. Based on these outcome data, the authors drew the conclusion that with this threshold value, NLR could be considered a diagnostic marker of prosthesis infection; in addition, it could also be employed over the follow-up period to document the patient's good therapeutic response. In our study, the choice of our threshold value set at 5 was probably too high. Most probably, we should have defined a lower threshold value to enable us to highlight the infected cases. Nevertheless, even with a threshold value of 2.45, the results of our study remain disappointing.

As in any scientific study, different types of errors likely occur and possibly influence the results, including random errors and systematic errors. In our study, several possible sources of random errors, or lack of precision, were possibly at the origin of an increase in the results' variability. First, a poorly calibrated or defective measuring instrument. While the measuring instruments were theoretically standardized, this could not be verified during our study. Second, the temporal variability of the measurements represented by the change of standard for the CRP threshold value among different patients. For some patients, CRP values were considered normal if  $<5$ , whereas for most of the patients, normality was defined by  $<1$ . Third, transcription errors in the database. The database has been established and applied conscientiously, but not yet electronically. Therefore, transcription errors may have occurred. Finally, two other sources of error should be noted in our study, including the imperfection of the reference test. Our Gold standard has been represented by the absence of clinical, radiological and biological signs after a two-year follow-up period. Of note, this Gold standard is not a completely reliable and accurate test, because some patients may present with an asymptomatic chronic infection; another reason is the systematic measurement of parameters were not always conducted at the same time for each patient. Indeed, the biological markers could have been measured up to 7 days before second-stage. Therefore, these parameters have not been completely evaluated in the same way among the patients.

Two main study strengths deserve to be highlighted. The first one is that there were no uncertain results. Indeed, there were no CRP and NLR results that could not be classified as positive or negative, as the tests studied consisted of quantitative biological markers, with the cut-offs set at a well-defined value for each parameter. The second strength is the long follow-up period. A two-year follow-up is long enough to allow

for the majority of infection recurrences to occur. Thus, this provides us with a better confidence in classifying the patient conditions as compared to shorter follow-ups.

## CONCLUSIONS

Currently, PJI research and new technologies mainly focus on the pathology's diagnostic aspect. Research is therefore particularly involved in identifying new biomarkers that can be considered diagnostic tests. However, only few studies have been conducted on diagnosing recurrent infection. In our study, we attempted to assess a possible predictive power of infection recurrence based on two biological markers: CRP and NLR. Nevertheless, measuring CRP and NLR, either individually or in combination, before second-stage revision arthroplasty turned out to be predictors of infection recurrence within two years of follow-up. An absolute test that would allow us to predict flawlessly, easily, and quickly the PJI control or absence of control during revision arthroplasty procedures is still lacking. Such a device could provide a rapid response to the surgeon; such a test could also be carried out extemporaneously upon prosthesis revisions. This tool should be independent of any antibiotic therapy if one wishes to use it to predict infection recurrence prior to second-stage prosthesis revision.

*Ethics:* This study was authorized by the Ethics Committee (Reference B403201523492) and has been performed with the participants' informed consent

*Source of Funding & Conflict of interest:* Source of funding for this study: none. Conflict of interest: none.

## REFERENCES

1. Deirmengian, C., Kardos, K., Kilmartin, P., Cameron, A., Schiller, K., & Parvizi, J. (2014). Diagnosing Periprosthetic Joint Infection: Has the Era of the Biomarker Arrived?. *Clinical Orthopaedics And Related Research*, 472(11), 3254-3262. <http://dx.doi.org/10.1007/s11999-014-3543-8>
2. Nodzo, S., Bauer, T., Pottinger, P., Garrigues, G., Bedair, H., & Deirmengian, C. et al. (2015). Conventional Diagnostic Challenges in Periprosthetic Joint Infection. *Journal Of The American Academy Of Orthopaedic Surgeons*, 23, S18-S25. <http://dx.doi.org/10.5435/jaaos-d-14-00385>
3. Ghanem, E., Azzam, K., Seeley, M., Joshi, A., & Parvizi, J. (2009). Staged Revision for Knee Arthroplasty Infection: What Is the Role of Serologic Tests Before Reimplantation?. *Clinical Orthopaedics And Related Research*, 467(7), 1699-1705. <http://dx.doi.org/10.1007/s11999-009-0742-9>

4. Deirmengian, C., Kardos, K., Kilmartin, P., Cameron, A., Schiller, K., & Parvizi, J. (2014). Combined Measurement of Synovial Fluid -Defensin and C-Reactive Protein Levels: Highly Accurate for Diagnosing Periprosthetic Joint Infection. *The Journal Of Bone & Joint Surgery*, 96(17), 1439-1445. <http://dx.doi.org/10.2106/jbjs.m.01316>
5. Forget, Patrice ; Moreau, Nicolas ; Engel, Harald ; Cornu, Olivier ; Boland, Benoît ; et. al. The neutrophil-to-lymphocyte ratio (NLR) after surgery for hip fracture (HF). In: *Archives of Gerontology and Geriatrics*, Vol. 60, no. 2, p. 366-371 (2015)
6. Gölge, UH., Kaymaz, B., Pazarcı, Ö., Kılınç, S., Öztemur, Z., Bulut, O. (2015). Neutrophil to Lym- phocyte Ratio May Be a Diagnostic Marker for Prosthetic Joint Infection. *J Clin Anal Med*. DOI: 10.4328/JCAM.3918.
7. Oschner, P., Borens, O., Bodler, P-M., Broger, I., Eich, G., Hefli, F., Maurer, T., Nötzli, H., Seiler, S., Suvà, D., Trampuz, A., Uçkay, I., Vogt, M., & Zimmerli, W. (2014). *Infections of the musculoskeletal system : Basic principles, prevention, diagnosis and treatment*. Granvaux, Switzerland: Swiss orthopaedics in-house publisher.
8. Ghanem, E., Azzam, K., Seeley, M., Joshi, A., & Parvizi, J. (2009). Staged Revision for Knee Arthroplasty Infection: What Is the Role of Serologic Tests Before Reimplantation?. *Clinical Orthopaedics And Related Research®*, 467(7), 1699-1705. <http://dx.doi.org/10.1007/s11999-009-0742-9>
9. Schindler, M., Christofilopoulos, P., Wyssa, B., Belaieff, W., Garzoni, C., & Bernard, L. et al. (2010). Poor performance of microbiological sampling in the prediction of recurrent arthroplasty infection. *International Orthopaedics*, 35(5), 647-654. <http://dx.doi.org/10.1007/s00264-010-1014-8>
10. Bilgen, O., Atici, T., Durak, K., Karaeminogullari, O., Bilgen, MS. (2001). C-reactive protein values and erythrocyte sedimentation rates after total hip and total knee arthroplasty. *J Int Med Res* 29:7-12.
11. Thienpont, E., Grosu, I., Jonckheere, S., & Yombi, J. (2012). C-reactive protein (CRP) in different types of minimally invasive knee arthroplasty. *Knee Surgery, Sports Traumatology, Arthroscopy*, 21(11), 2603-2610. <http://dx.doi.org/10.1007/s00167-012-2345-3>
12. Yombi, J., Schwab, P., & Thienpont, E. (2015). Neutro-phil-to-lymphocyte ratio (NLR) distribution shows a better kinetic pattern than C-reactive protein distribution for the follow-up of early inflammation after total knee arthroplasty. *Knee Surgery, Sports Traumatology, Arthroscopy*, 24(10), 3287-3292. <http://dx.doi.org/10.1007/s00167-015-3921-0>
13. Gölge, UH., Kaymaz, B., Pazarcı, Ö., Kılınç, S., Öztemur, Z., Bulut, O. (2015). Neutrophil to Lym- phocyte Ratio May Be a Diagnostic Marker for Prosthetic Joint Infection. *J Clin Anal Med*. DOI: 10.4328/JCAM.3918.