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Neutrophil-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

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Abstract
Purpose This study aimed to assess whether the neutrophil-to-lymphocyte ratio (NLR) distribution could have a better kinetic pattern than C-reactive protein (CRP) distribution to evaluate early post-operative inflammation after total knee arthroplasty (TKA).
Methods A prospective study was performed on 587 patients. CRP and NLR were collected pre-operatively and at post-operative days 2, 4, 21 and 42. Mean peak values and distribution were compared between CRP and NLR.
Results Mean CRP levels were 163, 161, 9 and 7 mg/L, respectively, at days 2, 4, 21 and 42. Mean NLR levels were 5, 3.5, 2.6 and 2.5, respectively, at days 2, 4, 21 and 42. At days 21 and 42, 20 % (102/503) and 21 % (93/433) of patients had not reached normal CRP levels. At day 21, there were 4.5 % (23/503) of patients with a NLR > 5 and 1 % (5/503) with an NLR > 10. At day 42, there were 5.5 % (24/433) of patients with an NLR > 5 and 0.7 % (3/433) with an NLR > 10.
Conclusion NLR has a faster normalization than CRP. It is potentially a better biomarker to follow post-operative inflammation or early infection after TKA.

Level of evidence II

Keywords Total knee arthroplasty · C-reactive protein · Neutrophil-to-lymphocyte ratio · Inflammation

Introduction

Total knee arthroplasty (TKA) provides pain relief, improves function, stability and mobility of the knee joint and reestablishes the normal axis of the lower limb. However, it remains a traumatic surgery, which induces inflammation with an acute-phase response. The acute-phase response comprises the nonspecific physiological and biochemical responses to most forms of infection, inflammation, malignant neoplasia and tissue damage. It is capable of controlling tissue damage, killing infectious organisms and inducing a repair process in order to restore host function [17]. Surgical trauma activates macrophages and monocytes that release pro-inflammatory cytokines. These cytokines include tumour necrosis factor alpha (TNF-α) and interleukin-1 beta (IL-1β), which are primarily responsible for the nonhepatic manifestations of the acute-phase response, including fever and tachycardia [17]. Pro-inflammatory cytokines stimulate the production and release of other cytokines, including interleukin-6 (IL-6) from macrophages, which is a primary effector in the production of acute-phase nonspecific proteins.

C-reactive protein (CRP), named as such for its capacity to precipitate the somatic C-polysaccharide of *Streptococcus Pneumonia*, is one of these acute-phase proteins. It is a pentameric protein found in blood plasma. C-reactive protein is synthesized in the liver by the hepatocytes and is under transcriptional control by cytokine IL-6 secretion [11, 19]. The normal concentration in healthy human

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adult serum is usually lower than 10 mg/L. However, following TKA, values may increase to more than 400 mg/L [22]. CRP is a common and inexpensive test to screen for systemic inflammation and the presence of prosthetic joint infection. CRP will never be diagnostic on its own and can only be interpreted with full knowledge of all other clinical and pathological results. C-reactive protein levels rise approximately 4–12 h after surgery and peak after 24–72 h [24]. Subsequently, C-reactive protein levels remain elevated for approximately 2 weeks in the immediate post-operative phase and reach normal levels 3–6 weeks after surgery. Some authors published that elevated CRP after TKA can persist for 150 days [10]. The inconvenience of a persistent high CRP level after TKA is that without clear clinical symptoms of infection, the positive predictive value of this test remains limited.

It has been demonstrated that surgery and sepsis lead to post-operative changes in white blood cell levels. Surgical trauma increases neutrophil blood levels via cytokines [18]. The polymorphonuclear (PMN) neutrophil progenitor is stimulated by IL-6 and IL-8, which attracts neutrophils to the wound site [6]. On the other hand, the lymphocyte count decreases by surgery-induced immunodepression. Under stressful conditions, such as trauma and sepsis, macrophages and monocytes are triggered to produce and release prostaglandin E2 (PGE_2). PGE_2 is capable of deactivating monocytes, thereby generating a significant decrease in the total systemic lymphocyte count [17]. While neutrophils are well recognized as infection markers, clinicians are less familiar with absolute lymphocytopenia as a possible marker for infectious diseases. Recently, the latter showed its potential in predicting bacteremia or the severity of several infectious diseases [23]. Combining both parameters seems a logical step, and the ratio of neutrophil over lymphocyte count is increasingly being used in several clinical circumstances. Neutrophil-to-lymphocyte ratio (NLR) is an easy, cheap, widely available, robust and convenient biomarker of the inflammatory response and has been shown to predict outcomes in oncology, digestive and cardiovascular diseases and haematogenous infections [2, 16, 21].

An NLR < 5 has been considered normal in the post-operative setting [7, 8]. An NLR of >10 was shown to have a high prognostic accuracy for predicting bacteremia [5]. To the best of our knowledge, no authors have studied the normal distribution pattern of NLR after TKA and its predictive value for post-operative complications. The clinical importance of this study would be to find another user-friendly biomarker that has a faster return to normal values than CRP and that could be used to monitor post-operative inflammation and early infection.

The hypothesis of this study was that after determination of the normal distribution pattern of NLR and comparison with the normal CRP distribution, it would be possible to see whether the NLR kinetic pattern would allow a better follow-up of early inflammation than the CRP kinetic pattern.

Materials and methods

This was a prospective observational study of patients with a diagnosis of primary knee osteoarthritis (OA) undergoing minimally invasive TKA in a university hospital. The period of recruitment was from 2007 to 2014. All knee arthroplasties performed at our institution are in a prospective database with blood tests performed pre-operatively, as well as at days 2, 4, 21 and 42. The data were retrieved from the operating software for patients' medical records of our institution (Medical Explorer v3r30b7, Saint-Luc Hospital, 2015). Analysis of CRP was performed using an immunoturbidimetric technique on an auto-analyser (Olympus). The assays display a limit of detection of 0.14 mg/L with the normal programme. Results were obtained at one decimal precision. Leucocyte count was typically included in the routine pre-operative and post-operative evaluation and assessed in our bio-clinical laboratory. All blood samples were anticoagulated by EDTA and processed in a blood analyser used in our laboratory [Sysmex (TOA Medical Electronics, Kobe, Japan)] for the determination of the complete blood cell counts and differential counts of leucocytes. The neutrophil-to-lymphocyte ratio was calculated on the basis of recorded absolute total neutrophil and lymphocyte counts (dividing the number of the former by the number of the latter) coming up with a numerical value.

The inclusion criterion was tricompartmental osteoarthritis without prior open surgery except meniscectomy. Exclusion criteria were patients with clinical signs of infection, neoplasia, inflammatory diseases (RA, Crohn, HIV…) or anyone who had an operative procedure within 3 months before admission. Patients with pre-operative C-reactive protein levels >10 mg/L were also excluded. Patients who developed a prosthetic joint infection or any other inflammatory or medical complication during the 6-week course of the study were also excluded. The aim of this study was to document the normal distribution pattern of NLR. Five hundred and eighty-seven patients were included in this study because of having blood tests available at day 2 and day 4. From these 587, 503 underwent blood tests at 21 days and 433 at day 42. These data were used for analysis.

The following demographic data were collected for each TKA patient: age, sex, BMI, type of anaesthesia and ASA score. Demographics are given in Table 1.

Table 1 Characteristics of the study group

	Number	Mean	SD	Minimum	Maximum
Age	587	70	10	26	92
BMI	587	30	5.6	16	43
Sex					
Men	170	7F/3M			
Women	417				
Laterality					
Left	280				
Right	307				
ASA score					
ASA 1	18				
ASA 2	486				
ASA 3	83				

BMI body mass index, *SD* standard deviation, *ASA* American Society of Anesthesiologists

Surgical technique

General anaesthesia was used for all surgeries. Cephalosporin was given 30–60 min before tourniquet inflation, which was released immediately after implantation of all components. A mini-para-patellar approach without eversion of the patella and without femorotibial dislocation was utilized. Intramedullary alignment was used on the femoral side and extra-medullary alignment for the tibia. The femoral hole was plugged in every TKA. The same cemented prosthesis with resurfacing of the patella (Vanguard PS, Biomet, Warsaw, IN, USA) was implanted. No drains were used. The same surgeon (ET) operated all patients.

The rehabilitation programme was identical for all patients, with full weight bearing without crutches along with active range of motion exercises the day after the operation. Thrombosis prevention was done with low molecular weight heparin (LMWH) for 10 days after surgery with either enoxaparin or nadroparin. All patients were examined every day during their hospitalization by the internist (JCY) and at the outpatient's clinic on days 21 and 42 by the surgeons. All patients initially included in this study were followed for at least 1 year, both clinically and radiologically, in order to detect the development of early prosthetic joint infection or other complications.

CRP and NLR values were determined pre-operatively and on days 2, 4, 21 and 42 post-operatively. CRP and NLR do not have the same units. The results of each were presented as a percentage with the highest value considered being 100 %.

All patients were informed of the data collection and their future anonymous statistical analysis. Our institutional ethics committee stated that a written consent is not needed for analyses of anonymized databases concerning data coming from routine practice, as permitted by national and European laws. Consequently, institutional ethical committee approval was granted for this study, and the committee approved the consent procedure. Before the blood sample was drawn, the study nurse discussed orally with the patient and asked permission to record the patient's data in our file. The institutional ethical committee gave its authorization for the retrospective analysis of the data (N°B403201 111 562 CEBH of the Université Catholique de Louvain, Brussels, Belgium).

Statistical analysis

Statistical analyses were performed using the Statistical Package for Social Science software (SPSS), version 21.0, for Windows (SPSS Inc., Chicago, IL, USA). Sample characteristics are presented as numbers, means, standard deviation and ranges. Categorical variables are presented as frequencies and percentages. The normal distribution of the data was assessed using the Kolmogorov–Smirnov test. The nonnormally distributed data were analysed using the nonparametric statistical Mann–Whitney test for independent samples and Wilcoxon signed-rank test for dependent samples. Comparison of observed proportions was performed using Chi-square and Fisher's exact test. A p value of < 0.05 was considered significant.

Results

The results for mean (SD) CRP levels are presented in Table 2. The mean pre-operative level was 3.3 mg/L. The highest mean peak of CRP was at day 2 (163.2 mg/L). At day 42, the CRP level was lower than 10 mg/L but not normalized (7.1 mg/L). Furthermore, on days 21 and 42, 20 % (102/503) and 21 % (93/433) of patients had not yet reached normal CRP levels (CRP < 10 mg/L).

The results for mean (SD) NLR levels are presented in Table 2. The mean pre-operative level was 2.6. The highest mean peak of NLR level was at day 2 (5). Already at day 4, the NLR began to decrease (3.6). At day 21, it was already normalized (2.6). At day 21, there were 4.5 % (23/503) of patients with an NLR > 5 and 1 % (5/503) with an NLR > 10. At day 42, there were 5.5 % (24/433) of patients with an NLR > 5 and 1 % (3/433) with an NLR > 10.

The comparison of the results of CRP and NLR expressed in percentage is presented in Fig. 1. The highest mean peak for both was at day 2. When comparing the pre-operative value and day 2 value, CRP increased more than NLR (50 times versus 2 times). NLR began its normalization earlier than CRP and recovered its baseline faster.

Table 2 Mean (SD) CRP and NLR levels over 6 weeks after TKA

	CRP (mg/L)			NLR		
	Mean	SD	Range	Mean	SD	Range
Pre-op	3.3	2.4	1.0–10.0	2.6	2.0	0.0–26.1
Day 2	163.2	76.7	10.0–498.0	5.0	2.8	0.8–27.9
Day 4	161.0	76.0	11.0–504.0	3.6	2.1	0.5–18.6
Day 21	9.0	19.0	1.0–228.0	2.6	1.8	0.3–24.2
Day 42	7.1	7.7	1.0–57.0	2.6	2.0	0.7–34.4

CRP C-reactive protein, *NLR* neutrophil-to-lymphocyte ratio, *SD* standard deviation, *Pre-op* pre-operative

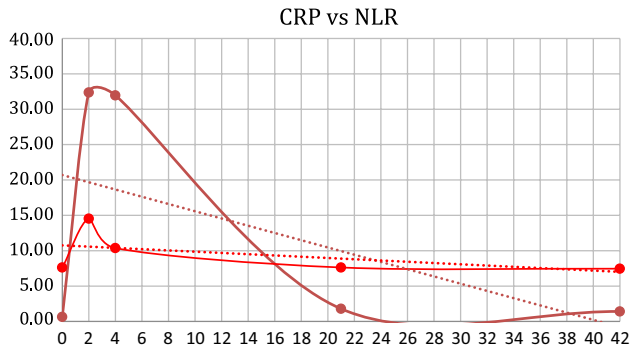

Fig. 1 Comparison of the kinetic of CRP (*high curve*) and NLR (*low curve*) distribution over 6 weeks after TKA. *CRP* C-reactive protein, *NLR* neutrophil-to-lymphocyte ratio, *Pre-op* pre-operative

Discussion

The most important finding of this study was that NLR showed a distribution pattern with a faster return to normal values than CRP in a standard post-operative period after TKA. The normal value for NLR should be <5, and in case of complications, a value of >10 is observed more than 3 weeks after TKA.

Plasma CRP levels reach their maximum values between the second and third post-operative days after elective knee arthroplasty, followed by a progressive decrease, with CRP usually achieving normal levels 3–6 weeks after surgery [3, 14]. Park et al. [20] described the time CRP takes to return to normal, with a rapid increase until day 2 and a subsequent decreasing pattern in 2 phases. The first phase is a gradual decrease to less than the normal reference level on the 42nd day; in the second phase, CRP returns to the pre-operative levels on the ninetieth day. Some authors published that elevated CRP after TKA can persist after 150 days [10]. In a study about minimal invasive total knee arthroplasty, Thienpont et al. [22] showed that median CRP values were almost normal on days 21 (7 mg/L) and 42 (4 mg/L); however, 34.5 % and 21.5 % of patients, respectively, had not reached normal CRP levels. Yombi et al. [24] confirmed these observations. In this current study, similar results are presented with 20 % of patients not recovering values under 10 mg/L at day 21 and 21 % at day 42. Mean CRP values were under 10 mg/dL, but had not yet reached their pre-operative levels at days 21 (9.0 mg/L) and day 42 (7.1 mg/L). The problem of this slow normalization of the distribution pattern of CRP is that in case of a suspected low-grade infection, CRP values will not help the clinician in his diagnostic algorithm and could trigger, in physicians unfamiliar with normal CRP distribution patterns, unnecessary, expensive and dangerous investigations for the patient or lead to unnecessary surgery [12]. In case the CRP was not normalized yet, looking at the NLR can help the surgeon decide what to do in this clinical situation.

In their study, Katoh et al. [13] demonstrated that the neutrophil level after total joint arthroplasty significantly rose from day 1, peaked at day 3 and decreased significantly following day 5. In this study, similar observations were made. Mean neutrophil level increased rapidly at day 2 and decreased at day 4. At day 21, the mean neutrophil level reached its pre-operative baseline. The same pattern was noted for the mean lymphocyte level. It decreased rapidly at day 2 and increased at day 4 to reach its normal level at day 21. The NLR, which is based on neutrophil and lymphocyte levels, behaved in the same way in our study. NLR had already recovered its pre-operative levels at day 21 (2.6). This observation suggests that NLR is normalized before day 21, therefore gradually decreasing between days 4 and 21. In the absence of blood samples between days 4 and 21, it was impossible to determine exactly at what day NLR reached its normal level. Nevertheless, the highly negative slope of the NLR between days 2 and 4 suggests that the NLR level recovered its baseline rapidly after day 4. In a study by de Jager et al. [5], the authors showed that an NLR > 10 has a higher prognostic accuracy in predicting bacteremia. In our study, 1 % had an NLR > 10 at day 21 and 1 % at day 42.

This study has some limitations. One of the limitations lies in the fact that all the patients did not do their blood tests up to day 42. At 21 and 42 days, the main reason for lack of blood tests was that the patient simply forgot. In that case, if the blood test from the previous visit was normal or an excellent clinical result was observed, it was not

proposed to repeat it. However, 74 % (433/587) of the subjects had all 4 blood tests performed.

Another limitation is that the normal value of NLR is not known and that it is unclear whether this value varies with age. Unpublished data from our hospital shows that out of 700 employees without active disease, 90–95 % had an NLR between 3 and 5. Therefore, considering a normal NLR value under 5 seems appropriate. NLR > 5 has been associated with poor prognosis in several diseases, including cardiovascular pathology [1, 4, 7, 15]. Bozbay et al. [4] reported the association of admission NLR values with in-hospital and long-term clinical outcomes in patients with infective endocarditis. Their study population was divided into tertiles according to their admission NLR values. The high-NLR group ($n = 76$) was defined as having an NLR value in the third tertile (NLR > 5.5) and the low-NLR group ($n = 95$) as having a value in the lower 2 tertiles ($\leq$5.5). In-hospital heart failure, haemodialysis, ventricular arrhythmia, septic shock and inotropic drug usage were more frequent in the high-NLR group. Patients in the high-NLR group had a higher incidence of in-hospital mortality (39.4 vs 18.9 %, $P = 0.003$). Furthermore, a high NLR value was an independent predictor of in-hospital mortality (hazard ratio, 2.53; 95 % confidence interval). In another study performed by Baglam et al. [1] (cutoff value of NLR was determined to be 5.4), the sensitivity and specificity of NLR for predicting complications associated with acute bacterial tonsillitis were 96 % and 83 %, respectively. The positive and negative predictive values of NLR were 84.96 % and 95.4 %, respectively. The potential use of NLR to predict severity of infection was confirmed by Loonen et al. [15] in their study where NLR was higher in bacteremic patients than in nonbacteremic patients, as were procalcitonin (PCT) and soluble urokinase plasminogen activator receptor (suPAR) levels. Gürol et al. [9] in a retrospective study included 1468 patients with suspected bacteremia and sepsis. Patients were grouped according to the following PCT criteria: levels <0.05 ng/ml (healthy group), 0.05–0.5 ng/ml (local infection group), 0.5–2 ng/ml (systemic infection group), 2–10 ng/ml (sepsis group) and >10 ng/ml (sepsis shock group). They found that an NLR value $\geq$5 might be a more convenient marker than CRP, due to its superior ability to detect bacterial infections at a lower cost. They also confirmed a correlation between the mean PCT level and NLR in patients with suspected infection in all study groups.

The strength of this study is that results were available for both NLR and CRP allowing to compare both biochemical markers. All patients underwent the same type of surgery by the same surgeon and had blood tests at the same intervals.

The results of this study describing the normal kinetics of NLR after TKA allow surgeons to include this new parameter when evaluating inflammatory complications or early infection after TKA. An NLR value over 10 after more than 3 weeks following surgery should be considered pathological and potentially suggests early infection of the joint if compatible with the clinical image.

Conclusion

NLR seems to be an easy and cheap biomarker to be included in the follow-up after total knee arthroplasty because it has a rapid return to normal values in the absence of complications. Furthermore, if it remains high, it is undoubtedly a sign of an underlying inflammatory or infectious problem. Further studies have to be done to learn more about NLR, especially for the detection of early periprosthetic joint infection following knee arthroplasty.

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Author contributions PES, JCY and ET designed the study protocol, collected the data, searched the literature, performed the statistical analysis and wrote the paper.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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