Determining the Stress Biomarker Profile in Patients Undergoing Total Knee Replacement and the Relationship with Outcome at 12 months

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All authors were fully involved in the study and preparation of the manuscript and the work has not been and will not be submitted for publication elsewhere.

Abstract

Background

Total knee replacement (TKR) is the commonest joint arthroplasty procedure worldwide. Despite excellent outcomes, some studies have reported dissatisfaction in up to 20% of patients.

There is evidence of an association between the biochemical stress response to surgery and outcomes. The objective of this study is to describe the stress biomarker profile for TKR, and correlate this with patient outcomes.

Methods

A prospective cohort study of 50 patients undergoing primary TKR was conducted. Serum IL-6, TNF-α, neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) were measured immediately pre- and post-operatively, and on Day 1 and 3. Follow up questionnaires including the Oxford Knee Score (OKS) and EuroQol five dimensions (EQ-5D) were completed at 12-months.

Univariate analysis was completed using a linear regression model ($p<0.05$).

Results

Serum IL-6, NLR, and PLR all increased to Day 1 post operatively, and decreased by Day 3. TNF-α values increased across all time points.
Statistical analysis found a significant negative correlation (r = -0.414; p=0.005) between pre-operative IL-6 and 12-month OKS. There was a significant positive correlation between pre-operative NLR and 12 month OKS (r = 0.272; p=0.039) and 12 month EQ5D (r = 0.268; p=0.043).

Conclusion

This is the first study to describe the biochemical stress response to TKR. The results raise the potential for a pre-operative risk stratification tool for patients based on IL-6 and NLR measurements.
Further research should be conducted to explore the underlying mechanisms involved, and investigate interventions to reduce pre-operative physiological stress with a view to improving post-operative outcomes.

Keywords

Total knee replacement; outcome; physiological stress response; stress biomarkers

1. Introduction:

Total Knee Replacement (TKR) is the most common joint arthroplasty procedure in the world(1). In England and Wales there are over 100,000 knee replacement procedures (2) performed each year at an estimated annual cost of £550 million to the NHS (1). These figures are set to significantly increase by 2030, due to a higher demand for TKR from an increasingly ageing population(3).

Although outcomes from TKR are generally excellent, rates of patient satisfaction post-operatively have been consistently reported as less than 85% in the literature, with some studies stating up to 20% of patients are unsatisfied with their knee replacement (1, 4). This accounts for up to 20,000 patients each year who are unsatisfied with the result of an expensive operation with the sole purpose of improving their quality of life. Consequently, this has been acknowledged by the James Lind Alliance, who highlighted the question: 'What pre-operative, intra-operative and post-operative factors can be modified to influence outcome following hip and knee replacement', as a research priority in 2014(5).

Efforts have been made at every level of the patient pathway to improve the situation, with previous studies focussing on pre-operative factors, including patient factors and pre-operative expectation (6, 7). Surgical factors, such as type of prosthesis and patient psychological well-being (6) have also been investigated. Despite extensive research, these factors do not account for the variability observed(8), and there is a need to identify additional factors that influence outcome. It is important to recognise those patients who are at risk of having a poor outcome, so that both clinician and patient can identify and appraise risks and benefits of the

procedure. It may also be possible for these patients to have additional, targeted interventions to optimise their post-operative outcome.

The biochemical stress response after surgery has been reported by a number of studies, particularly the trends of Interleukin-6 (IL-6), C-Reactive Protein (CRP) and Neutrophil-to-Lymphocyte ratio (NLR)(9, 10). These have been used to monitor the post-operative inflammatory response, acting as markers of post-operative complications and infection. Surgical trauma activates macrophages and monocytes that release pro-inflammatory cytokines, which increase white blood cell numbers, especially neutrophils(11). Research has shown that patients with an increase in leukocyte numbers early intra-operatively, and a decrease later during surgery, show enhanced recovery(12). Individuals that displayed this more ‘adaptive’ leukocyte redistribution had better post-operative functional outcome scores after meniscectomy(12). In tumour resection surgery, an elevated preoperative NLR has been shown to correlate with a poorer prognosis(13).

Within the context of joint arthroplasty, the literature examining the relationship between the biochemical stress response and patient outcomes is limited. Whilst studies have explored the rise and fall of biomarkers such as CRP, IL-6 and NLR, this has been in the context of a diagnostic tool in identifying post-operative infection (10, 11). The stress response utilising other markers (TNF-alpha, Platelet-to-Lymphocyte ratio (PLR)) have remained largely undefined.

Furthermore, there is a lack of data that correlates the effect of the stress response to post-operative outcomes.

2. Objectives:

The primary aim of the study is to describe the physiological stress biomarker profile for total knee replacement.

The secondary objective is an exploratory analysis to measure the association between the stress response and post-operative outcomes.

3. Material and methods:

3.1 Study design and participants

We carried out a prospective cohort study, consisting of patients undergoing primary TKR for osteoarthritis (OA) at University Hospitals of Coventry and Warwickshire (UHCW) NHS Trust. Patients were eligible if they were >18 years old and undergoing primary total knee replacement for osteoarthritis. Exclusion criteria included: (i) patients undergoing revision surgery of an existing knee implant, (ii) bilateral TKR, (iii) uni-compartmental knee replacement, (iv) pre-existing inflammatory conditions such as rheumatoid arthritis, (v) an acute infection, (vi) pregnancy and (vii) concurrent use of immunosuppressive agents. Patients who required hospital admission in the previous 3 months due to a surgical or medical problem were also excluded.

All patients received routine antibiotic prophylaxis as per the trust protocol. Operations were performed by consultant Orthopaedic surgeons in the trust who perform knee arthroplasty as part of their routine practice.

The study was approved by the Health Research Authority (HRA; IRAS project ID: 212309).

3.2 Study protocol

All patients undergoing a TKR at UHCW were invited to enter the study. Patients received a letter of invitation and information sheet, as well as a personal invitation via a telephone call from a member of the research team. A member of the research team obtained informed, written consent. Patients were permitted to withdraw from the study at any time without giving reason.

Following enrolment, relevant baseline and demographic data was collected from patients via posted questionnaires. Pre-operative blood tests were taken at induction of anaesthesia, and post-operatively in the theatre recovery room.

Further blood samples were collected on Day 1 and Day 3 post-operatively. If a patient was discharged prior to Day 3, only Day 1 samples were collected and still included in the study.

All collected samples were analysed and stored in the UHCW Laboratory.

3.3 Follow-up

Patients were followed up at twelve-months post-operatively. Post-operative patient reported outcome measures (PROMs) were measured using the Oxford Knee Score (OKS) (14) and the EuroQol five dimensions' questionnaire (EQ-5D) (15).

3.4 Blood biomarkers

The biomarkers chosen for analysis in this study were based on a literature search and include Interleukin 6 (IL-6), Tumour necrosis factor alpha (TNF-α), Neutrophil-to-lymphocyte ratio (NLR) and Platelet-to-lymphocyte ratio (PLR).

3.5 Statistical analysis

A power calculation was not completed for this exploratory study. In keeping with published, prospective, exploratory studies investigating biomarkers (6,8,9), a sample size of 50 patients was deemed suitable, and allowed for linear regression analysis.

Data was stored and graphs generated using Microsoft Excel. Univariate analysis using a linear regression model was completed using the Statistical Package for Social Science software (SPSS; SPSS Inc., Chicago, IL, USA). The dependent variables were 12-month OKS and EQ5D, and the independent variables were the biomarkers at different time points. A p-value of <0.05 was considered significant. Missing data was handled using the pairwise method.

4. Results

Among the patients undergoing primary TKR at UHCW between January and October 2017, 50 patients were recruited for the study. The average age was 69.3 years, with 20 females and 30 male patients. The mean pre-operative OKS was 17.1 and EQ5D was 64.3.

In total 45 patients completed the 12 month OKS and 44 completed the EQ5D questionnaires, giving follow up rates of 90% and 88% respectively. The results for mean OKS, EQ5D, and blood biomarkers are summarised in Table 1. The mean 12-month OKS was 31.8, and EQ5D was 66.9. Serum IL-6, NLR, and PLR all increased to Day 1 post operatively, and decreased by Day 3. TNF-α values gradually increased across the time points and continued to rise at day 3. Blood biomarker trends are depicted in Figures 1-4; the error bars represent standard error of the mean.

The univariate regression analysis found a significant negative correlation (Pearson correlation coefficient, r = -0.414; p=0.005) between pre-operative IL-6 and final OKS at 12 months post-operatively. The coefficient was -0.1529, which implies that 1 standard deviation change in IL-6 results in a change of 6 in OKS. There was also a significant positive correlation between pre-operative NLR and 12 month OKS (r = 0.272; p=0.039) and 12 month EQ5D (r = 0.268; p=0.043). There were no significant correlations identified between TNF-α and PLR with PROMs, or post-operative biomarkers and 12 month outcomes. Results from the regression analysis are summarised in Table 2.

5. Discussion:

This exploratory study is the first of its kind to describe the biochemical stress response to TKR. According to the times of sample collection in this study, serum IL-6, NLR, and PLR were all highest at Day 1 post operatively, and decreased by Day 3, whereas TNF-α values gradually increased during the same period. These trends

are consistent with the literature on the stress response reported after other surgical procedures, which generally describe that cytokine levels peak at 24 hours post-operatively and remain elevated for 48-72 hours(11).

The main finding of this study is a significant negative correlation between pre-operative IL-6 and Oxford Knee Score at 12 months ($p=0.005$). The minimal clinically important difference (MCID) for OKS in total knee replacement has been reported as 5.0 in the literature(14). We have reported that one standard deviation increase in pre-operative IL-6 resulted in a decrease of 6 in OKS, which is greater than the MCID, and hence highlights the clinical relevance of our results. There was also a significant positive correlation between pre-operative NLR and 12-month OKS ($p=0.039$) and EQ5D ($p=0.043$). The correlation between pre-operative IL-6 and 12 month EQ5D ($p=0.055$) may become significant with a greater number of cases. This is an exploratory study and if these findings are replicated in other centres, then the potential is to utilise IL-6 and NLR as a pre-operative risk-stratification tool for patients undergoing knee replacement surgery. Those with elevated IL-6 or a low NLR could be appropriately consented and made aware of the possibility of a poorer 12-month outcome, and hence would not have unrealistic expectation from the procedure. Another potential avenue that could be explored if these results are supported by future studies is whether any interventions can be developed to optimise patients pre-operatively, using IL-6 or NLR as markers of effectiveness, or whether the mechanism behind these relationships can be identified and addressed.

The value of IL-6 for monitoring inflammation after major surgery has been shown in several studies(9). Brocca et al(15) showed that persistent elevation of IL-6 postoperatively had a good predictive value for 30-day and overall mortality in cardiac surgery ($P<0.05$). They suggested therefore that an inflammatory biomarker

panel may be useful in the workup for patients undergoing cardiac surgery. This can be replicated in the pre-operative workup of knee arthroplasty patients, if our findings are corroborated by further research.

Furthermore, Fink-Neuboeck et al(16) conducted a prospective clinical trial that demonstrated the positive impact of IL-6 as a predictive biomarker in the early diagnosis of postoperative systemic inflammatory response syndrome (SIRS) after major thoracic surgery. Serum IL-6 increased significantly on the day of operation and preceded the median onset of postoperative SIRS, which occurred the next day ($P<0.01$). The relationship between IL-6 and post-operative complications after knee arthroplasty was not investigated by this study, and is another potential subject for future research.

Yombi et al(10) conducted a prospective study comparing the measurement of NLR and CRP post operatively in TKA as markers of early inflammation, and concluded that NLR was a more effective biomarker owing to its faster normalization rate. Lieto et al(17) reported that patients with a high pre-operative NLR and low Lymphocyte-to-monocyte ratio had a greater recurrence rate after gastric cancer surgery compared to those with opposite results. However, in their study Lieto et al measured serum NLR one week pre-operatively, whereas in our study the sample was collected at induction of anesthesia immediately before the operation. Since it has been already shown that serum NLR has a fast normalization rate (9), there is a possibility that the NLR results recorded in our study were influenced by pre-operative psychological or physiological stresses(18). Further research is warranted to investigate this phenomenon.

There are no other studies to our knowledge that investigate the impact of pre-operative blood biomarker results on post-operative outcomes, or describe the post-

operative physiological stress response to arthroplasty surgery, and hence this highlights the importance of our findings.

Our results should however be treated with caution. Our measurement of pre-operative blood biomarkers was taken at induction of anaesthesia, which in reality leaves insufficient time for any pre-operative intervention. Future studies should take measurements at longer intervals pre-operatively, when patients are in a stable state and there is sufficient time to implement appropriate interventions. Our study has several other limitations. Notably, our sample consisted of a greater proportion of males (60%) than females (40%), which is against the normal trend in TKR surgery where females predominate (2). Furthermore, there is a small sample size, and missing data particularly at certain time points. A power calculation was not deemed necessary for this exploratory study; hence the results should be considered in light of the small sample size and the data can be used to inform larger future studies. Although there was missing data, particularly on Day 3 due to early discharges or unwillingness to have further blood tests, and some missing baseline PROMs data due to variable patient compliance with questionnaires, 84% of patients had baseline bloods data, and 12-month follow up in the study was 90% for the OKS and 88% for EQ5D. We consider this to be a good follow up rate, and therefore we can be reasonably confident in the relationships observed between the pre-operative measures and 12-month PROMs.

Furthermore, whilst our selection of blood biomarkers is consistent with those reported in the literature, there are a selection of other markers such as cortisol that have been investigated in other surgical procedures and hence may warrant further review in knee arthroplasty. We also did not take into account history of cancer or

chronic infection in the subjects recruited, which could potentially influence the stress response.

In summary, our study found significant correlations between pre-operative serum IL-6 and NLR level with Oxford Knee Score at 12 months, and pre-operative NLR with 12-month EQ5D, after total knee replacement. Despite the limitations of the study, this finding raises the possibility that the pre-operative IL-6 and NLR measurements can be used as a screening and risk stratification tool for total knee replacement patients. This opens avenues to develop novel approaches to reduce pre-operative physiological stress, and develop interventions that may improve outcomes in the future.

Future research should be conducted to investigate the relationship between pre-operative IL-6 and NLR with post-operative outcomes, ideally in the form of an adequately powered prospective study with measurements at longer intervals pre-operatively, further exploration of the underlying mechanisms involved, and long term follow up of patients.

Declaration of interest

None.

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Table 1:

		Number, N	***Mean (SD)***
IL-6 pg/ml	Pre-operative	42	17.9 (39.7)
	Recovery	39	22.3 (52.5)
	Day 1	36	47.0 (68.4)
	Day 3	29	17.1 (36.8)
TNF-a pg/ml	Pre-operative	42	2.18 (0.933)
	Recovery	39	2.35 (0.922)
	Day 1	34	2.28 (0.929)
	Day 3	30	2.84 (2.65)
NLR	Pre-operative	48	2.72 (2.38)
	Day 1	49	9.15 (5.21)
	Day 3	34	6.25 (3.52)
PLR	Pre-operative	48	183 (102)
	Day 1	49	210 (101)
	Day 3	34	144 (123)
OKS	Pre-op	35	17.1 (8.11)
	12 months	45	31.8 (13.3)
EQ5D	Pre-op	33	64.3 (22.2)
	12 months	44	66.9 (21.8)

Table 2:

	Pre-op		*Post-op*		*Day 1*		*Day 3*	
OKS	***r***	***p***	***r***	***p***	***r***	***p***	***r***	***p***
IL-6	**-0.414**	**0.005**	-0.228	0.097	-0.045	0.403	-0.098	0.316
TNF-a	-0.091	0.294	-0.220	0.106	0.196	0.150	0.096	0.317
NLR	**0.272**	**0.039**			-0.013	0.466	0.196	0.141
PLR	0.132	0.200			0.165	0.143	0.240	0.093
EQ5D								
IL-6	-0.267	0.055	-0.090	0.305	-0.004	0.491	-0.224	0.141
TNF-a	0.089	0.299	-0.034	0.424	0.240	0.105	0.211	0.150
NLR	**0.268**	**0.043**			0.027	0.432	0.161	0.194
PLR	0.160	0.156			0.205	0.093	0.126	0.249

Captions:

Table 1: Blood biomarker and PROMs results

Table 2: Summary of univariate analysis with OKS and EQ5D as the dependent variables (r = Pearson correlation coefficient; $p<0.05$)

Figure 1: Mean serum concentrations of IL-6

Figure 2: Mean serum concentrations of TNF-a

Figure 3: Mean serum NLR

Figure 4: Mean serum PLR