Technology and Health Care 32 (2024) 585–593
DOI 10.3233/THC-220635
IOS Press

Impact of the blood group on postoperative CRP and leukocyte levels after primary total hip and knee arthroplasty

Philipp Egenolf[a,*], Christopher Wahlers[a], David Grevenstein[a], Birgit S. Gathof[b], Peer Eysel[a] and Johannes Oppermann[a]
[a]*Department for Orthopaedic and Trauma Surgery, Faculty of Medicine, University Hospital Cologne, University of Cologne, Cologne, Germany*
[b]*Institute of Transfusion Medicine, Faculty of Medicine, University Hospital Cologne, University of Cologne, Cologne, Germany*

Received 10 October 2022
Accepted 11 August 2023

Abstract.
BACKGROUND: C-reactive protein (CRP)- and leukocyte levels are common parameters to evaluate the inflammatory response after orthopaedic surgery and rule out infectious complications. Nevertheless, both parameters are vulnerable to disturbing biases and therefore leave room for interpretation.
OBJECTIVE: Since blood groups are repeatedly discussed to influence inflammatory response, our aim was to observe their impact on CRP and leukocyte levels after total hip and knee arthroplasty (THA/TKA).
METHODS: Short term postoperative CRP and leukocyte levels of 987 patients, who received either primary TKH ($n = 479$) or THA ($n = 508$), were retrospectively correlated with their blood group. ABO, Rhesus and a combination of both blood groups were differentiated.
RESULTS: CRP levels after TKA were significantly higher in blood type AB than in type A and O on day 2–4 and also than in type A on day 6–8. Leukocyte levels after THA were significantly higher in blood group type O than in type A on day 6–8 while still remaining in an apathological range. We observed no significant differences between Rhesus types and Rhesus types and CRP or leukocyte levels.
CONCLUSION: We observed significantly increased CRP levels after TKA in patients with blood group AB. Since the elevated CRP levels do not account for early periprosthetic infection, surgeons should include this variation in their postoperative evaluation.

Keywords: Arthroplasty, leukocytes, C-reactive protein, ABO blood-group system

1. Introduction

Total hip and total knee arthroplasty (THA and TKA) belong to the most frequently performed proce-

*Corresponding author: Philipp Egenolf, Department for Orthopaedic and Trauma Surgery, University Hospital of Cologne, Kerpener Str. 62, 50937 Cologne, Germany. E-mail: philipp.egenolf@uk-koeln.de.

dures in orthopaedic surgery [1]. With increasing numbers of implantation, the number of complications has risen as well [2,3]. Among these, periprosthetic joint infections (PJI) play a major role accounting for one third of all revision operations [4].

Coming along with a high burden for affected patients, PJI often requires interdisciplinary treatment and long-term antibiotic therapy following mostly at least one surgical revision [5].

As it's diagnostic delay affects the outcome negatively, early identification of PJI is crucial [4,5,6].

Apart from clinical assessment, inflammatory blood parameters play a dominant role to detect first signs of PJI [5,6]. Therefore they are commonly monitored in perioperative management.

Leukocyte levels as well as C-reactive protein (CRP) levels are the most frequently used laboratory parameters due to their high sensitivity and its inexpensiveness and wide spread availability [6,7]. Nevertheless, interpretation of these parameters can be challenging.

Irrespective of its clinical importance, CRP represents an unspecific inflammation parameter. Produced in the hepatocytes, elevated CRP levels can be observed in many different diseases and situations e.g. malignoma, acute and chronic infection, rheumatic inflammation and trauma [8]. Equally raised levels of leukocytes can be found in the named settings but they can also increase by specific medication (e.g. steroids), malignoma such as leukaemia, tobacco smoking and many more [9]. Postoperatively increased blood levels of CRP and leukocytes are observed on a usual basis, decreasing after the major surgery within the first postoperative week [10,11].

Recently, Zhao et al. [12] raised attention to the topic of the influence of blood groups (BG) on the susceptibility of infectious diseases. Although being disproved by Latz et al. [13], the influence of BGs on the host's increasing or decreasing susceptibility to many types of viral and microbiological infection has already been targeted before [14,15,16,17]. As an example, BGs play a role play a role as a (co-)receptor for parasites, viruses like hepatitis B and C, or bacteria, e.g. Treponema pallidum and Staphylococcus aureus [18]. Furthermore, blood group antigens ease the intracellular signal transduction, adhesion or uptake and modify the congenital immune answer to infection [19]. Primary orthopaedic diseases like knee osteoarthritis also seem to be influenced by BGs, although their impact is controversially discussed [20].

Anyhow, the correlation between BGs and the named inflammatory blood parameters has not been investigated in neither the septical nor aseptical spectrum yet. The influence of BGs on the perioperative development of these parameters would be of value to improve its interpretation and therefor early identification of PJI. If specific blood groups would correlate to higher apathological CRP levels, their elevation after surgery could rather be tolerated.

Since TKA and THA are representative major orthopaedic operations, we correlated perioperative CRP- and leukocyte-levels of patients with TKA and THA with their BGs to address this issue. Among all 41 different BGs and their alleles known, the most commonly used in clinical practice are ABO and Rhesus blood groups. Since keeping blood reserves for every arthroplasty is part of clinic's routine quality management, ABO and Rhesus blood groups are regularly identified before TKA and THA.

We had postulated, that there is no significant difference in leukocyte and CRP-Levels depending on the patient's BGs regarding the ABO- and Rhesus-system.

2. Materials and methods

This study was approved by the institutional review board of the medical faculty of the University of Cologne (Ethical committee study number 20-1710; approved on January 10th, 2021) and complies with the Declaration of Helsinki. Due to the retrospective nature of this investigation, written consent was waived. All blood samples were clinically indicated and not performed for study purposes.

2.1. Investigated data

For the present study we retrospectively identified 1015 patients, who underwent primary THA or TKA between 2013 and 2018 due to high grade osteoarthritis. Exclusion criteria were rheumatic diseases, revision-procedures, chronic infections and partial joint replacements.

Of those patients we excluded all, who underwent revision surgery within the first 12 months after primary surgery.

In conclusion, we analysed the data of 508 THA-recipients (227 male, 280 female) with a mean age of 65.8 years (Min. 14.7 years, Max. 95.8 years, $\pm$ 14.4 years) and 479 TKA-recipients (193 male, 286 female) with a mean age of 66.6 years (Min. 28.0 years, Max. 89.1 years, $\pm$ 10.4 years).

2.2. Data

Following data were collected from the clinical intern electronic archive:

- Sex
- Age at date of surgery
- Type of surgery (TKA/THA)
- C-reactive protein (CRP) in mg/l in the serum and leukocytes in the blood per μl
- Blood group (BG) according to the ABO (A/B/AB/O) and Rhesus system (Rh+/ Rh−)

Besides preoperative and imminent postoperative blood-examination, further timepoints varied slightly due to a change of postoperative examination protocol in our clinic within the studied years. Additional blood examination was occasionally indicated by clinical evaluation.

We therefore formed four time periods, in which measurements were grouped:

- preoperative (one to three days before operation)
- day 1 (first postoperative day)
- day 2–4 (second to fourth postoperative day)
- day 6–8 (sixth to eighth postoperative day)

CRP-measurements were performed according to standard procedures of this hospital's Institute for laboratory medicine using a third generation Latex-test in an automated process (Cobas C 702, Roche Diagnostics, Mannheim, Germany). Until 2016, the lower limit for detection of CRP, was defined as $<$ 3 mgl/l, since 2016 the lower limit was defined as $<$ 0.6 mg/l, the standard value was $<$ 5 mg/l. For statistical analysis, values were equalled at the lower detection limit at the time of measurement.

Leukocytes were counted with an automated analysing-system (Sysmex XN-9100 and Sysmex XN-1000, Sysmex, Norderstedt, Germany), the standard value was 4.4–11.3/μl.

ABO Blood Group type and Rhesus Blood Group was identified by the Institution of transfusion medicine, by standardized, accredited procedures (Bio-Rad IH-500 and Bio-Rad IH-1000 Blood Bank Testing System for Blood grouping ABO/D and Phenotyping Rh/K).

Through obtaining both ABO and Rhesus blood groups, we were able to analyse the differences not only within its' blood group system, but also in combination. By combining ABO and Rhesus system, eight subgroups were created and therefore compared to each other.

2.3. Statistical methods

Statistical analysis was performed using SPSS version 26.0 (SPSS Inc., Chicago, IL, USA). Non-parametric tests were used to detect statistically significant correlations. A p-value less than 0.05 was regarded statistically significant.

Table 1
Distribution (number of patients (n)) of each blood group among THA and TKA patients

	THA			TKA		
Blood-group	Rh+	Rh−	Total	Rh+	Rh−	Total
O	183	35	208	162	36	198
A	183	27	210	178	34	212
B	46	7	53	34	6	40
AB	24	3	27	24	5	29
Total	436	72	508	398	81	479

At first, we showed via Kolmogorov-Smirnov-Test, that none of the parameters followed a normal distribution.

Mann-Whitney U tests were applied to evaluate differences in CRP- and leukocyte values regarding Rhesus-factor.

Kruskal-Wallis tests were used to determine, if CRP- or leukocyte values differed significantly divided either by the ABO system or a combination of ABO and Rhesus system.

3. Results

3.1. THA

The patient's distribution regarding each blood group system is shown in Table 1. Average values of each blood group's CRP and leukocyte level are shown in Table 2.

3.2. THA – ABO system

Via Kruskal-Wallis test a significant difference between ABO blood types was only shown for leukocyte values on day 6–8 ($p = 0.023$). In the described time period leukocyte levels in patients with blood type O (average = 8,1/μl, $n = 206$) were significantly higher than in patients with blood type A (average = 7,7/μl, $n = 201$).

3.3. THA – Rhesus system

Mann-Whitney U test showed no significant differences between different Rhesus factors regarding leukocytes and CRP at any timepoint.

3.4. THA – Combination of ABO and rhesus system

Kruskal-Wallis test showed significant differences between the groups in leukocyte levels at the first postoperative day ($p = 0.03$) and the sixth to eighth postoperative day ($p = 0.044$). Anyhow, two-sided testing showed no significant differences for specific subgroups of blood group type ($p < 0.05$).

3.5. TKA

The distribution of the blood group systems among TKA patients is shown in Table 1. Average values of each blood group's CRP and leukocyte level are shown in Table 3.

Table 2
Average CRP (mg/l) and leukocyte levels (/μl) after THA depending on their blood group; the table reads as: **average**/standard deviation/quantity (n). Levels of significance (p-value) are given for comparison of blood groups *, Rhesus factor (**) and the combination of blood group and Rhesus factor (***)

Blood group		Preoperative				Day 1				Day 2–4				Day 6–8			
		CRP		Leukocytes		CRP		Leukocytes		CRP		Leukocytes		CRP		Leukocytes	
A	Rh+	**6.9**/20.7/209	**6.2**/16.6/182	**7.3**/2.2/209	**7.2**/2.1/182	**47.5**/33.4/192	**46.7**/31.3/168	**8.7**/2.5/195	**8.5**/2.5/170	**84.1**/52.0/172	**81.6**/52.1/150	**8.0**/2.7/171	**7.8**/2.6/149	**38.0**/32.8/200	**37.3**/32.8/173	**7.7**/4.6/201	**7.6**/4.8/174
	Rh−		**11.5**/38.7/27		**8.3**/2.0/27		**52.4**/45.7/24		**9.7**/2.2/25		**102.4**/48.6/22		**9.6**/2.7/22		**42.6**/33.1/27		**8.5**/3.2/27
B	Rh+	**9.0**/13.6/53	**8.8**/12.5/46	**7.2**/2.1/52	**7.1**/2.1/46	**54.7**/43.5/51	**51.4**/43.4/45	**8.6**/2.9/51	**8.4**/3.0/45	**94.7**/65.4/44	**89.5**/65.7/40	**8.1**/4.2/44	**8.1**/4.4/40	**36.8**/30.9/50	**36.2**/31.5/44	**7.7**/4.6/50	**7.6**/4.9/44
	Rh−		**9.7**/20.6/7		**7.9**/1.9/6		**80.1**/38.3/6		**9.8**/2.6/6		**147.0**/33.2/4		**8.7**/1.4/4		**41.1**/28.3/6		**8.5**/2.7/6
AB	Rh+	**7.2**/8.4/27	**7.8**/8.7/24	**7.6**/2.3/27	**7.6**/2.3/24	**59.6**/32.9/25	**58.2**/31.9/22	**9.4**/3.7/26	**9.1**/3.1/23	**100.2**/65.1/23	**97.6**/63.5/21	**8.0**/2.9/23	**7.9**/2.7/21	**39.8**/27.7/27	**39.3**/29.2/24	**7.7**/2.8/27	**7.7**/2.6/24
	Rh−		**2.6**/2.9/3		**7.8**/2.9/3		**70.4**/46.2/3		**11.7**/7.5/3		**127.8**/104.1/2		**8.8**/6.1/2		**44.0**/10.7/3		**8.0**/5.1/3
0	Rh+	**6.7**/20.8/216	**7.0**/21.9/181	**7.3**/2.4/216	**7.3**/2.5/181	**48.4**/27.4/200	**48.5**/28.5/167	**9.4**/3.0/203	**9.6**/3.0/170	**91.1**/50.0/176	**91.5**/49.3/152	**8.4**/2.9/178	**8.5**/4.5/154	**41.0**/32.7/205	**41.4**/33.2/173	**8.1**/2.3/206	**8.1**/3.1/175
	Rh−		**5.0**/7.2/35		**7.2**/1.8/135		**47.5**/23.1/33		**8.6**/2.8/33		**88.5**/57.4/24		**7.8**/3.1/24		**38.7**/34.5/32		**7.8**/2.0/31
p-value (*)		0.307		0.863		0.156		0.147		0.450		0.952		0.336		0.023	
p-value (**)		0.723		0.058		0.264		0.361		0.068		0.107		0.692		0.249	
p-value (***)		0.174		0.219		0.223		0.030		0.064		0.090		0.490		0.044	

Table 3
Average CRP (mg/l) and leukocyte levels (/μl) after TKA depending on their blood group; the table reads as: **average**/standard deviation/quantity (n). Levels of significance (p-value) are given for comparison of blood groups *, Rhesus factor (**) and the combination of blood group and Rhesus factor (***)

Blood group		Preoperative				Day 1				Day 2–4				Day 6–8			
		CRP		Leukocytes		CRP		Leukocytes		CRP		Leukocytes		CRP		Leukocytes	
A	Rh+	**4.4**/7.1/212	**4.7**/7.6/178	**7.2**/2.2/211	**7.2**/2.2/177	**64.4**/34.4/193	**63.5**/34.1/163	**9.4**/2.6/193	**9.4**/2.6/164	**109.6**/65.5/172	**110.1**/67.2/142	**8.3**/4.1/173	**8.5**/4.4/143	**42.6**/30.7/209	**42.6**/30.8/176	**7.5**/2.3/209	**7.5**/2.3/176
	Rh−		**3.0**/3.0/34		**7.1**/2.3/34		**69.1**/36.2/30		**9.4**/2.7/29		**107.6**/57.9/30		**7.8**/2.1/30		**42.9**/30.7/33		**7.2**/2.0/33
B	Rh+	**4.0**/6.2/40	**4.1**/6.7/34	**7.1**/1.7/40	**7.1**/1.5/34	**58.8**/30.2/34	**59.1**/32.2/28	**9.0**/2.8/34	**9.0**/2.6/28	**106.2**/53.7/32	**110.9**/57.3/26	**7.2**/2.5/32	**7.5**/2.2/26	**39.3**/22. 4/40	**41.6**/23.0/34	**7.2**/2.4/40	**7.1**/2.0/34
	Rh−		**3.3**/2.0/6		**6.7**/2.8/6		**57.5**/20.4/6		**9.0**/4.2/6		**85.7**/29.1/6		**7.8**/3.9/6		**26.8**/14.8/6		**7.7**/4.1/6
AB	Rh+	**5.7**/9.5/29	**6.8**/10.1/24	**7.5**/2.0/29	**7.4**/2.0/24	**77.5**/38.5/27	**79.2**/41.3/22	**10.5**/2.9/27	**10.4**/3.1/22	**164.8**/86.5/23	**160.9**/87.6/18	**8.6**/3.3/23	**8.7**/3.6/18	**65.5**/43.4/28	**62.6**/41.3/23	**8.3**/2.8/28	**8.3**/2.9/23
	Rh−		**0.2**/0.5/5		**7.8**/1.9/5		**69.9**/23.6/5		**10.5**/2.1/5		**179.0**/90.6/5		**8.4**/2.0/5		**79.0**/55.3/5		**8.1**/2.6/5
0	Rh+	**5.4**/8.9/198	**4.8**/5.7/162	**7.6**/2.6/195	**7.6**/2.7/159	**60.7**/31.5/179	**61.4**/32.8/148	**9.9**/3.0/181	**9.9**/3.2/149	**115.1**/67.9/161	**117.6**/71.7/131	**8.6**/2.8/161	**8.6**/3.0/131	**54.2**/43.5/186	**55.3**/45.9/151	**7.9**/3.3/186	**7.9**/3.4/151
	Rh−		**8.1**/17.0/36		**7.8**/2.2/36		**57.1**/24.7/31		**9.8**/2.4/32		**103.9**/47.2/30		**8.2**/2.1/30		**49.5**/31.6/35		**7.9**/2.6/35
p-value (*)		0.267		0.29		0.15		0.155		0.021		0.271		0.004		0.163	
p-value (**)		0.774		0.586		0.801		0.802		0.927		0.565		0.998		0.891	
p-value (***)		0.171		0.630		0.481		0.582		0.149		0.713		0.032		0.573	

3.6. TKA – ABO system

Via Kruskal-Wallis test, significant differences among CRP-levels on day 2–4 ($p = 0.021$) and day 6–8 ($p = 0.004$) were shown.

Pairwise comparison revealed significantly higher CRP-levels on day 2–4 of patients with blood group type AB (average = 164.8 mg/l, $n = 23$) than of type 0 (average = 115.1 mg/l, $n = 161$) ($p = 0.048$) and type A (average = 109.6 mg/l, $n = 172$) ($p = 0.013$).

On day 6–8 CRP-levels of type AB (average = 65.5 mg/l, $n = 28$) exceeded those of type A (average = 42.6 mg/l, $n = 209$) significantly ($p = 0.013$).

3.7. TKA – Rhesus system

There were no significant differences between Rh+ and Rh−, as analysed via Mann-Whitney U test at any timepoint.

3.8. TKA – Combination of ABO and rhesus system

Kruskal-Wallis test showed stand-alone significant differences in CRP-levels on day 6–8 ($p = 0.032$). However, evaluation of this difference through pairwise comparison showed no significant differences between two distinct groups.

3.9. Excluded patients with early revision

We excluded 13 patients with THA (n: 0+ = 3, A+ = 6, B+ = 2, AB+ = 1, AB− = 1) and 9 patients with TKA (n: 0+ = 3, 0− = 2, A+ = 2, B+ = 1, AB+ = 1), who underwent revision surgery due to PJI within the first 12 months after primary surgery. As a matter of further information, their blood groups were also identified as shown. Statistical evaluation was waived due to the low number of individuals.

4. Discussion

Our study was designed to evaluate differences between CRP and leukocyte blood levels after TKA and THA depending on ABO and Rhesus blood group type.

While differences in ABO types have been correlated to varying vWF and FVIII [21], the role of the ABO system on inflammation remains subject of current investigation and hasn't been solved in its entirety. As part of further understanding, separate aspects and factors of the inflammatory process have been investigated with its correlation to the ABO system. Hereby ABO-depending differences in the expression of H-antigen [22], IL-6 [23], TNF-Alpha [15] and ICAM-1 [16] have been shown.

On the orthopaedic field, ABO was linked to primary osteoarthritis (POA). While blood group AB showed to be associated with a higher risk of POA of the knee [20], blood group O had an inverse correlation with POA of the hip [24]. Further studies found ABO type to influence Postoperative Cognitive Dysfunction (POCD) after THA [25] and the risk of thromboembolism after total joint arthroplasty [26, 27].

Our study showed increased leukocyte levels in THA-patients with ABO-type O on day 6–8. It should be noted that both the elevated levels of type O as well as the low levels of type A are apathological, concerning a healthy interval of 4.5–11.0/ul. Therefore, this difference should not account for a clinical deduction.

CRP levels in TKA-patients with ABO-type AB were significantly higher on day 2–8. Li et al. found blood type AB to be associated with POA of the knee [20]. They found blood type AB to be associated with a lower synovial expression of H antigen. H antigen is related to Le^Y antigen, which is a proinflammatory factor. Since H antigen's and Le^Y's biosynthesis depends on same substrates [28], Li et al. hypothesised an inverse correlation between H antigen and Le^Y. Regarding our results, an increased Le^Y expression might also be the cause of the elevated CRP value in TKA patients type AB.

The difference in quantity was in parts a result of the retrospective nature of the study as well as of the ABO imbalance in the general population. Nevertheless, this difference between blood groups might have clinical importance, since it helps physicians to interpretate postoperative CRP level.

By superficial comparison of the blood groups of early revision patients, no prominent imbalance was noted. For that reason and due to the low number of cases, no statistical rested thesis can be made. Although the susceptibility to infections has been shown to be influenced by blood groups, a correlation between PJI and blood groups has not been under investigation, but would be of interest. A considerably higher number of patients is therefore required. The results of our study however do not allow for a recommendation regarding patient selection and there is no current literature supporting such a thesis.

By comparing Rhesus blood group type Rh+ and Rh− to another, our study found no statistical difference in inflammation parameters after TKA and THA. Our results are supported by the fact that yet there was no correlation described between the Rhesus blood group and the host's susceptibility to diseases and inflammation in the reviewed literature.

Limitations of our study include the observation of only CRP and leukocyte levels, as previously described findings showed differences in the inflammatory pathway depending on the blood group type. Our study also doesn't emphasize additional parameters such as obesity [29], ASA score [30] and smoking habits [9], which influence CRP and leukocyte level. Anyhow, our aim was to describe the impact of blood groups on standard blood examination parameters. For that reason, the limitations of the blood parameters (e.g. interleukin-6) were intentional, since they play no role in standard postoperative evaluation after TKA or THA. Further bias such as smoking habits remained uncovered due to the retrospective nature of our study. The second limitation is the selection bias of the included patients. Most patients were referred to our university hospital, because they were rejected in other hospitals due to pre-existing conditions like obesity, pulmonary or cardiac diseases. These conditions however are often associated with aseptically elevated CRP values [29,31]. The observed preoperative CRP values are therefore moderately above reference values. The final limitation of our study is the relatively small sample size in blood groups B and AB. Despite showing a profound difference in CRP values (TKA) of type AB, in total only 29 patients were part of this subgroup. Therefore, a sampling error cannot be ruled out.

To our knowledge, our study is the first to shine light on the relevance of ABO and Rhesus blood groups for the profound inflammatory blood parameters after THA and TKA. Since both are major orthopaedic surgeries, a relatively large patient collective was analysed. Furthermore, no studies focused on the blood group's impact on postoperative inflammation.

5. Conclusion

While differences of the leukocyte levels only arose in an apathological range, we observed significantly increased CRP levels after TKA in patients with blood group AB. Surgeons should know, that blood group AB might lead to higher CRP levels after major orthopaedic surgery. Since the elevated CRP levels did not account for early periprosthetic infection, this might be a symptom of a potentially varying general inflammatory response. Surgeons should therefore include this variation in their postoperative evaluation.

Acknowledgments

The authors have no acknowledgments.

Conflict of interest

The authors declare that they have no conflict of interest.

Funding

The authors report no funding.

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