

# Mildly decreased preoperative bilirubin levels are considered as risk factors for periprosthetic joint infection after total hip and knee arthroplasty

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## Research article

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# Abstract

## Background and Purpose

Many blood biomarkers are routinely assessed before joint arthroplasty, but only few are commonly used to guide surgeons in determining patients most at risk of periprosthetic joint infection (PJI). The objective of this study was to investigate the correlation between preoperative bilirubin level and PJI after hip and knee arthroplasty.

## Methods

A retrospective analysis was performed on patients undergoing revision hip and knee arthroplasty at our hospital from January 2016 to December 2019. Laboratory biomarkers were collected before the primary arthroplasty, as well as general patient information. The correlation between the above blood biomarkers and postoperative PJI was analyzed.

## Results

A total of 72 patients (30 hips/42 knees) were analyzed, including 39 patients with PJI and 33 patients without PJI. Except for total bilirubin (TB) and direct bilirubin (DB), there was no significant difference between the remaining laboratory biomarkers. The preoperative TB and DB in the PJI group were  $10.84 \pm 0.61 \mu\text{mol/L}$  and  $3.07 \pm 0.19 \mu\text{mol/L}$ , respectively, which were lower than those in the non-PJI group ( $14.68 \pm 0.75 \mu\text{mol/L}$  and  $4.70 \pm 0.39 \mu\text{mol/L}$ ,  $p = 0.0001$  and  $0.0002$ ). The AUC of preoperative TB to predict PJI was 0.7552 ( $p = 0.0002$ , cutoff =  $11.55 \mu\text{mol/L}$ , sensitivity = 66.67%, specificity = 75.76%, PPV = 76.47%, NPV = 65.79%). Meanwhile, the AUC of preoperative DB was 0.7603 ( $p = 0.0001$ , cutoff =  $4.00 \mu\text{mol/L}$ , sensitivity = 84.62%, specificity = 54.45%, PPV = 68.75%, NPV = 75%).

## Conclusions

The serum levels of TB and DB before the primary arthroplasty of PJI patients were lower than those non-PJI patients, and the preoperative values lower than  $11.55 \mu\text{mol/L}$  and  $4.00 \mu\text{mol/L}$  could be considered as risk factors for postoperative PJI.

## Introduction

Periprosthetic joint infection (PJI) is one of the catastrophic complications following joint arthroplasty that actually increases financial burden and suffering to the patients and their families [1]. PJI is the number one cause of failure in total knee arthroplasty (TKA) and the third leading cause of failure in total hip arthroplasty (THA) [2, 3]. The reported incidence of PJI is 1–3% following primary arthroplasty and 3–5% after revision arthroplasty [4, 5]. With prolonged life expectancy and a growing indication for primary

joint arthroplasty, there will be a huge increase in the number of PJI patients. Early and accurate identification of individuals at high risk of PJI bears on clinical decision-making and development of effectively preventive strategies.

Given the severity of PJI, previous studies have identified a tremendous number of risk factors for PJI [6–9]; they can be divided into the patient (intrinsic factor) and environment (extrinsic factor) and play a crucial part in pre-, intra- or post-operative period. Many blood biomarkers are routinely assessed before joint arthroplasty, but only few are commonly used to guide surgeons in determining patients most at risk of PJI [10]. Among these biomarkers are C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), D-dimer, fibrinogen, and other visceral organ specific biomarkers, which are mainly used to monitor or detect comorbidities, such as diabetes, inflammatory arthritis, renal disease, immunosuppression, and malnutrition. Since the abnormal values may be affected by the pre-existing comorbidities and misguide the surgeons' diagnosis for PJI.

Recently, mildly elevated bilirubin levels in adults have been reported to be protective against pathologies such as cardiovascular diseases, diabetes type 2, and several cancers, supposedly due to its powerful anti-inflammatory and anti-oxidative effect [11, 12]. Previous studies have found that bilirubin impairs bactericidal activity of neutrophils through scavenging reactive oxygen species (ROS) and increases NADPH oxidase-1 (NOX-1) and cyclooxygenase-2 (COX-2) in patients with hyperbilirubinemia, resulting in physiologic effects mitigated by increased antioxidant activity [13, 14].

Laky B et al [15] have reported that mildly decreased preoperative bilirubin levels with a cutoff at 8.72  $\mu\text{mol/L}$  or 0.51 mg/dL were significantly associated to patients with PJI after shoulder and knee arthroplasty. To our knowledge, there is no other study on the correlation between serum bilirubin levels and PJI. Therefore, our retrospectively case-control and large sample study was conducted to compare preoperative serum bilirubin levels between patients with and without PJI after hip and knee arthroplasties and to explore the hypothesis that patients with PJI compared to without PJI after THA and TKA would present with lower preoperative bilirubin levels.

## Materials And Methods

### Patients

After receiving approval from the institutional review board at our hospital, a retrospective analysis was performed on patients undergoing revision hip and knee arthroplasty at our center from January 2016 to December 2019 (Fig. 1). Those patients who also received primary arthroplasties in our hospital before revision arthroplasties were included in this study. And we also excluded patients undergoing primary arthroplasties with liver diseases, inflammatory and infectious arthritis, or incomplete data. Patients with PJI were diagnosed by the 2014 modified MSIS criteria in this current study [16]. Patients without PJI were defined as cases undergoing single-stage revision for a diagnosis other than infection (loosening, wear, instability, malalignment, adverse local tissue reactions, or other aseptic causes).

# Clinical and blood biomarkers

Demographic data including age, gender, body mass index (BMI), smoking habit, alcohol consumption; comorbidities such as obesity, diabetes mellitus, hypertension, cardiovascular, kidney, and thyroid diseases; indication for joint arthroplasty, pre-operative period of revision surgery, details regarding postoperative PJI and preoperative blood biomarkers including total bilirubin [TB (umol/L)], direct bilirubin [DB (umol/L)], alanine aminotransferase [ALT (U/L)], aspartate aminotransferase [AST (U/L)], alkaline phosphatase [ALP (U/L)], glutamate-pyruvate transaminase [GGT (U/L)], creatinine (umol/L), serum glucose (mmol/L), serum sodium (mmol/L), serum potassium (mmol/L), hemoglobin (g/L), RBC count ( $10^{12}/L$ ), WBC count ( $10^9/L$ ), blood platelet count ( $10^9/L$ ), CRP (mg/L), interleukin-6 [IL-6 (pg/ml)], ESR (mm/h), activated partial thromboplastin time [APTT (S)], fibrinogen (g/L) and D-dimer (ug/ml) were collected, analyzed, and compared between the two groups. The serum TB and DB in our hospital were detected by vanadate oxidation method and fully automatic biochemical analyzer.

## Statistical analysis

All statistical analyses were performed using SPSS Statistics 22.0 (IBM® Corporation, Armonk, NY, USA). Patients' general data was presented using descriptive statistics. Categorical data was presented by numbers and quantitative data as means with standard deviation (SD) or range. For continuous and normal distributed data, the Student's t-test was applied and the Mann-Whitney U test was used for ordinal or non-normally distributed data to determine differences between two groups. A Pearson Chi-square or Fisher's exact test was performed to analyze categorical variables. A receiver operating characteristic (ROC) curve analysis was constructed and used to determine a possible cut-off point for preoperative bilirubin to distinguish between PJI and non-PJI patients. The area under the ROC curve (AUC) was assessed to better evaluate the diagnostic accuracy of preoperative bilirubin. An AUC of one represents an ideal test with 100% sensitivity and 100% specificity; whereas an area AUC < 0.5 indicates that the diagnostic test is less useful. A conditional logistic regression model of the case-control study was used to further assess the bilirubin cut-off. Odds ratio (OR) and corresponding 95% confidence intervals (CI) were calculated.

## Results

### General information

Includable were 39 patients with PJI after primary THA (n = 13) or TKA (n = 26), compared with the contemporaneous 33 patients without PJI after primary THA (n = 17) or TKA (n = 16). The differences between two groups in age, gender, BMI, affected joints, smoking habit, alcohol habit and preoperative comorbidities were of no statistical significance, except for the time from primary arthroplasty to revision surgery (Table 1). The duration in patients with and without PJI were  $107.3 \pm 28.25$  weeks and  $399.7 \pm 49.46$  weeks, respectively ( $p < 0.0001$ ).

Table 1  
Comparisons of general information and comorbidities between cases and controls

	Cases with PJI (n = 39)	Controls without PJI (n = 33)	P value
Age	58.59 ± 2.44	55.61 ± 2.60	0.4062
Gender	21 F/18 M	16 F/17 M	0.6502
BMI (kg/m <sup>2</sup> )	28.61 ± 1.84	25.67 ± 0.72	0.1678
Hip/Knee	13 Hip / 26 Knee	17 Hip / 16 Knee	0.1189
Time to revision (W)	107.3 ± 28.25	399.7 ± 49.46	<b>&lt; 0.0001</b>
Smoking habit	2	2	0.8634
Alcohol habit	5	3	0.6158
Diabetes mellitus	3	3	0.8306
Hypertension	5	4	0.9288
Cardiovascular disease	6	4	0.6899
Thyroid disease	2	1	0.6571
ASA classification	I 1 / II 34 / III 4	I 4 / II 27 / 2 III	0.2330
BMI, Body mass index; ASA, American Society of Anesthesiologists			

## Comparisons of preoperative blood biomarkers

All comparisons between patients with and without PJI regarding preoperative blood biomarkers are presented in Table 2. The only preoperative biomarkers significantly different between the PJI and non-PJI group were TB and DB. The preoperative TB in patients with and without PJI were 10.84 ± 0.61 µmol/L and 14.68 ± 0.75 µmol/L, respectively (p = 0.0001). While the preoperative DB were 3.07 ± 0.19 µmol/L and 4.70 ± 0.39 µmol/L in PJI group and no-PJI group (p = 0.0002).

Table 2  
Comparisons of preoperative blood biomarkers between cases and controls

	Cases with PJI (n = 39)	Controls without PJI (n = 33)	P value
Total bilirubin (µmol/L)	10.84 ± 0.61	14.68 ± 0.75	<b>0.0001</b>
Direct bilirubin (µmol/L)	3.07 ± 0.19	4.70 ± 0.39	<b>0.0002</b>
ALT (U/L)	20.54 ± 1.97	20.53 ± 2.97	0.9973
AST (U/L)	19.65 ± 1.90	19.27 ± 2.45	0.9024
ALP (U/L)	78.67 ± 5.13	79.38 ± 8.82	0.9431
GGT (U/L)	32.42 ± 3.36	37.07 ± 11.39	0.6721
Creatinine (µmol/L)	65.69 ± 2.75	64.23 ± 2.04	0.6824
Serum glucose (mmol/L)	5.23 ± 0.27	5.26 ± 0.24	0.9305
Serum sodium (mmol/L)	141.10 ± 0.46	142.90 ± 0.55	0.1562
Serum potassium (mmol/L)	3.84 ± 0.05	3.92 ± 0.07	0.3605
6 (g/L)	136.20 ± 1.72	140.70 ± 2.84	0.1573
RBC count (10 <sup>12</sup> /L)	4.47 ± 0.05	4.54 ± 0.09	0.5004
WBC count (10 <sup>9</sup> /L)	6.04 ± 0.27	6.18 ± 0.35	0.7309
Blood platelet count (10 <sup>9</sup> /L)	210.50 ± 8.85	207.50 ± 7.81	0.8051
CRP (mg/L)	0.43 ± 0.07	0.49 ± 0.09	0.5569
IL-6 (pg/ml)	6.59 ± 2.44	4.88 ± 1.08	0.7115
ESR (mm/h)	10.27 ± 1.16	10.78 ± 1.34	0.7727
APTT (S)	35.80 ± 0.79	35.47 ± 1.44	0.8337
Fibrinogen (g/L)	3.24 ± 0.16	3.28 ± 0.18	0.8608
D-dimer (µg/MI)	0.71 ± 0.13	0.53 ± 0.11	0.5427
ALT, Alanine transaminase; AST, Aspartate transaminase; ALP, Alkaline phosphatase; GGT, γ-glutamyl transferase; RBC, Red blood cell; WBC, White blood cell; CRP, C-reactive protein; IL, Interleukin; ESR, Erythrocyte sedimentation rate; APTT, Activated partial thromboplastin time.			

The AUC for the preoperative TB levels to distinguish between PJI and non-PJI patients was 0.7552 (95%CI: 0.6448–0.8657, p = 0.0002; Fig. 2) and the cut-off value for a maximum of sensitivity and specificity was a preoperative TB level of 11.55 µmol/L (sensitivity: 66.67%, specificity: 75.76%, positive predictive value [PPV]: 76.47%, negative predictive value [NPV]: 65.79%). The AUC for the preoperative DB levels to determine between PJI and non-PJI patients was 0.7603 (95%CI: 0.6510–0.8696, p = 0.0001;

Fig. 3) and the cut-off value for a maximum of sensitivity and specificity was a preoperative TB level of 4.00  $\mu\text{mol/L}$  (sensitivity: 84.62%, specificity: 54.45%, PPV: 68.75%, NPV: 75%).

According to conditional regression analysis, lower preoperative bilirubin levels (TB < 11.55  $\mu\text{mol/L}$  or DB < 4.00  $\mu\text{mol/L}$ ) were significantly associated as a predictor for PJI (OR: 6.25, 95%CI: 2.21 to 17.65 or OR: 6.60, 95%CI: 2.18 to 19.98).

## Discussion

For this retrospective and case-control study, we evaluated preoperative blood biomarkers of 39 patients with PJI controlled to 33 patients without PJI after THA and TKA. The two groups were comparable in matching general data (including age, gender, joint types), as well as the potential risk factors (BMI, smoking habits and alcohol use), and other comorbidities. The biggest finding is that that preoperative bilirubin levels were significantly lower in patients with PJI compared to controls without PJI after THA and TKA.

In 2018, Parvizi J et al [17] conducted the study of an evidence-based and validated criteria and updated version of the PJI diagnostic criteria. According to research results, the new criteria demonstrated a sensitivity of 97.7% and specificity of 99.5%. This is not to say that the diagnosis of PJI is particularly easy, in fact, the diagnostic process for PJI involved a multi-pronged and stepwise approach evaluating blood, synovial fluid, and tissue specimen tests. CRP and ESR are supported by strong evidence a useful “ruling out” tests [18, 19]. Synovial fluid tests such as leukocyte count and neutrophil percentage, leukocyte esterase,  $\alpha$ -defensin, cultures, and next-generation sequencing for microorganism can play an important diagnostic role [20–22]. If preoperative evaluation with serum and synovial fluid tests does not secure a diagnosis, then the frozen section tissue histopathology may help make the diagnosis [23].

Multiple risk factors were identified to be associated with PJI, including characteristics of the patient, surgical procedure and postoperative care [24]. A predictive model is a statistical equation that predicts an individual’s disease risk based on a combination of the values of multiple risk factors. Risk prediction models first originated in the area of cardiovascular disease prevention and have been widely used globally in clinical and public health practice [25]. And many predictive models for postoperative PJI have been developed [6]. Del Toro MD et al [26] developed and validated baseline, perioperative and at-discharge risk-scoring systems for PJI in patients undergoing arthroplasty. And they found that factors associated with PJI in the perioperative stage were THA, rheumatoid arthritis, obesity, National Nosocomial Infections Surveillance (NNIS) index  $\geq 2$ , significant wound bleeding and superficial surgical site infection. However, available risk models to predict PJI have been developed using poor methodology and have several limitations, it needs further validation using new data and its clinical effectiveness should be evaluated using a RCT design.

According to the research findings of Kunutsor SK et al [6], only one predictive model that was mainly based on invasive data such as CRP, ESR and microbial etiology. In this current study, the preoperative CRP and ESR levels were  $0.43 \pm 0.07$  and  $10.27 \pm 1.16$  in PJI group and  $0.49 \pm 0.09$  and  $10.78 \pm 1.34$  in

non-PJI group, respectively ( $p = 0.5569$  and  $p = 0.7727$ ). Many blood biomarkers are routinely assessed before joint arthroplasty, but it is unaware of these biomarkers as possible predictors for postoperative PJI.

In this study, the most significant and new finding were that with mildly elevated preoperative bilirubin levels ( $TB \geq 11.55 \mu\text{mol/L}$  or  $DB \geq 4.00 \mu\text{mol/L}$ ) were less prone to PJI, which was consistent with the research result of Laky B [15]. However, the research by Laky has some limitations, including a small sample study with only 18 PJI patients (8 shoulders and 10 knees), only analyzing the total bilirubin and the definition of PJI was not the classic criteria. And we believe that mildly lower bilirubin, especially within normal levels, has potential to serve as a predictive factor for postoperative PJI and thus, should be further investigated. For clinical application, mildly to moderate elevated bilirubin levels without signs of inflammation and increased liver biomarkers, bilirubin can be seen as protective factor for postoperative PJI. The AUC of preoperative TB to predict PJI was 0.7552 with sensitivity of 66.67% and specificity of 75.76%, meanwhile the AUC of preoperative DB to predict PJI was 0.7603 with sensitivity of 84.62% and specificity of 54.45%.

Many epidemiological studies reported that higher bilirubin levels were in connection with reduced mortality and the protective role of bilirubin were explained by its anti-oxidative and anti-inflammatory capacities [12, 27]. The exact mechanisms are still little-known. Previous research has explored the possible anti-inflammatory effect of bilirubin and found bilirubin might inhibit the production of pro-inflammatory cytokines (e.g. IL-6), which are in turn responsible for CRP production in the liver tissue [11, 12, 28]. And other studies with different pathologies such as cardiovascular diseases [29], metabolic syndrome and type 2 diabetes [30], cerebrovascular diseases [31], osteoporosis [32], and even in rheumatoid osteoarthritis [33] also showed negative correlations with bilirubin concentrations. This has also been reported in studies evaluating the association between bilirubin and bacterial infections or associated models such as pathogen exposures including endotoxin, although not all studies show protective effects. And the bilirubin levels were increased by stimulating heme oxygenase activity in animal model [34]. Therefore, it was speculated that increasing bilirubin levels within the normal range preoperatively in patients with arthroplasties has potential protective role by its anti-oxidative and anti-inflammatory capacities, which would decrease the risk for PJI.

The current study has several limitations. First, this was a retrospective and single center study. Second, the study sample was 72 patients (39 patients with PJI and 33 patients without PJI). Thus, prospectively multi-center study with larger sample was need to validate our finding. Third, mechanisms behind the potential protective effects of the mildly elevated bilirubin levels in this cannot be drawn. We are also aware that the difference in bilirubin between PJI group and non-PJI group was small and that the mildly decreased bilirubin levels within normal serum bilirubin ranges in the PJI group can only be regarded as relative to the control group and not relative to normal serum values. However, according to our research results, bilirubin seems a promising preoperative and easy available factor, which might be able to predict postoperative PJI.

# Conclusions

In summary, this retrospective study demonstrated that the levels of TB and DB before the primary replacement of PJI patients were lower than those non-PJI patients and decreased bilirubin levels below a cut off at TB = 11.55  $\mu\text{mol/L}$  or DB = 4.00  $\mu\text{mol/L}$  could be considered as risk factors for postoperative PJI after THA and TKA.

# Declarations

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## Availability of data and supporting materials

The datasets supporting the conclusions of this article are included within the article and its supplementary materials.

## Ethics approval

This study was approved by the ethics committee of the Chinese PLA General Hospital.

## Author contributions

Jun Fu, Xiyue Chen and Jiying Chen carried out the study, participated in data collection and drafted the manuscript. Chi Xu, Ming Ni and Wei Chai performed the statistical analyses and were involved in its design. Xiang Li, Libo Hao and Yonggang Zhou participated in acquisition, analysis or interpretation of data. All authors read and approved the final manuscript.

## Conflicts of interest

The authors declare no conflict of interest.

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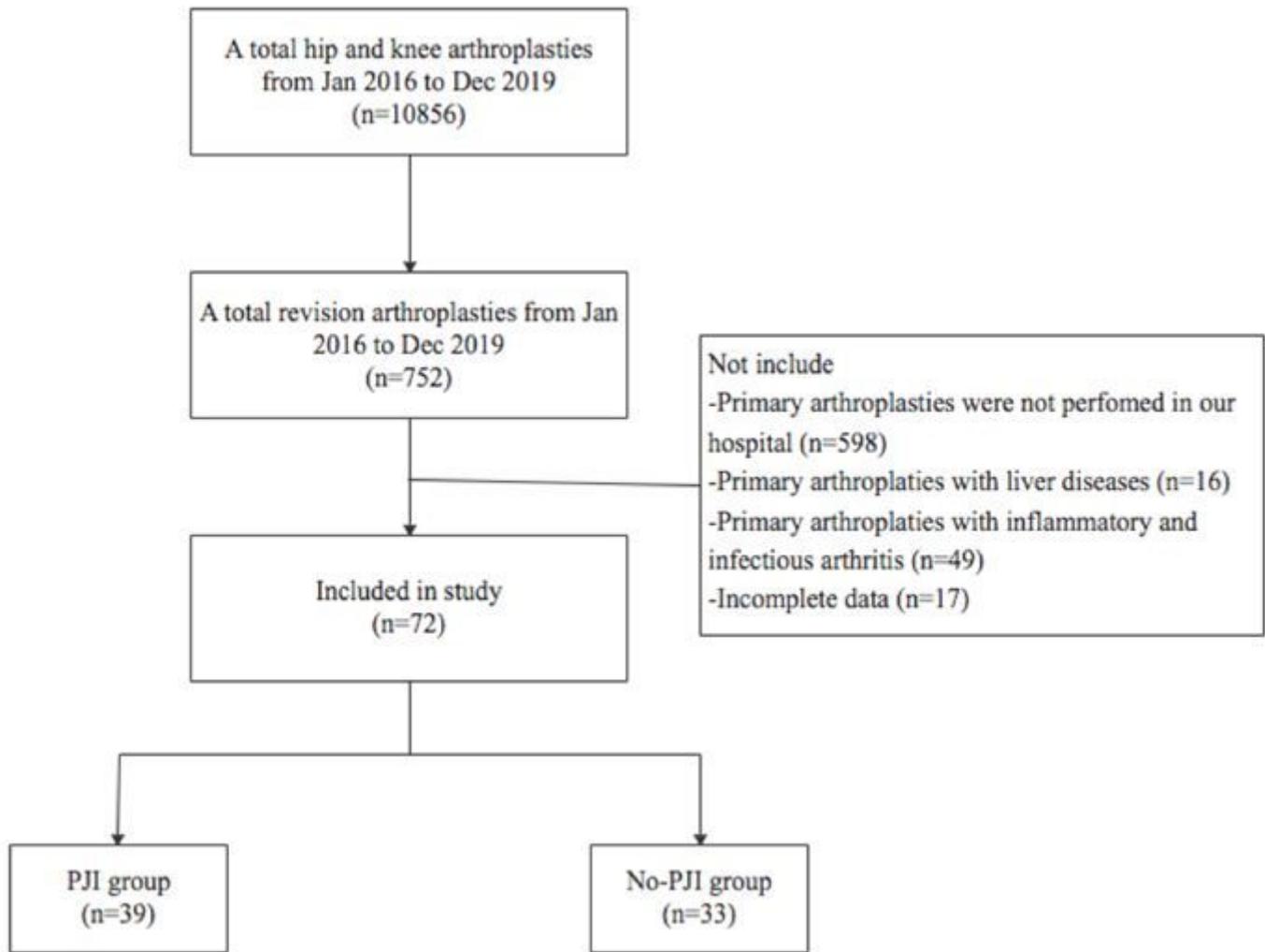
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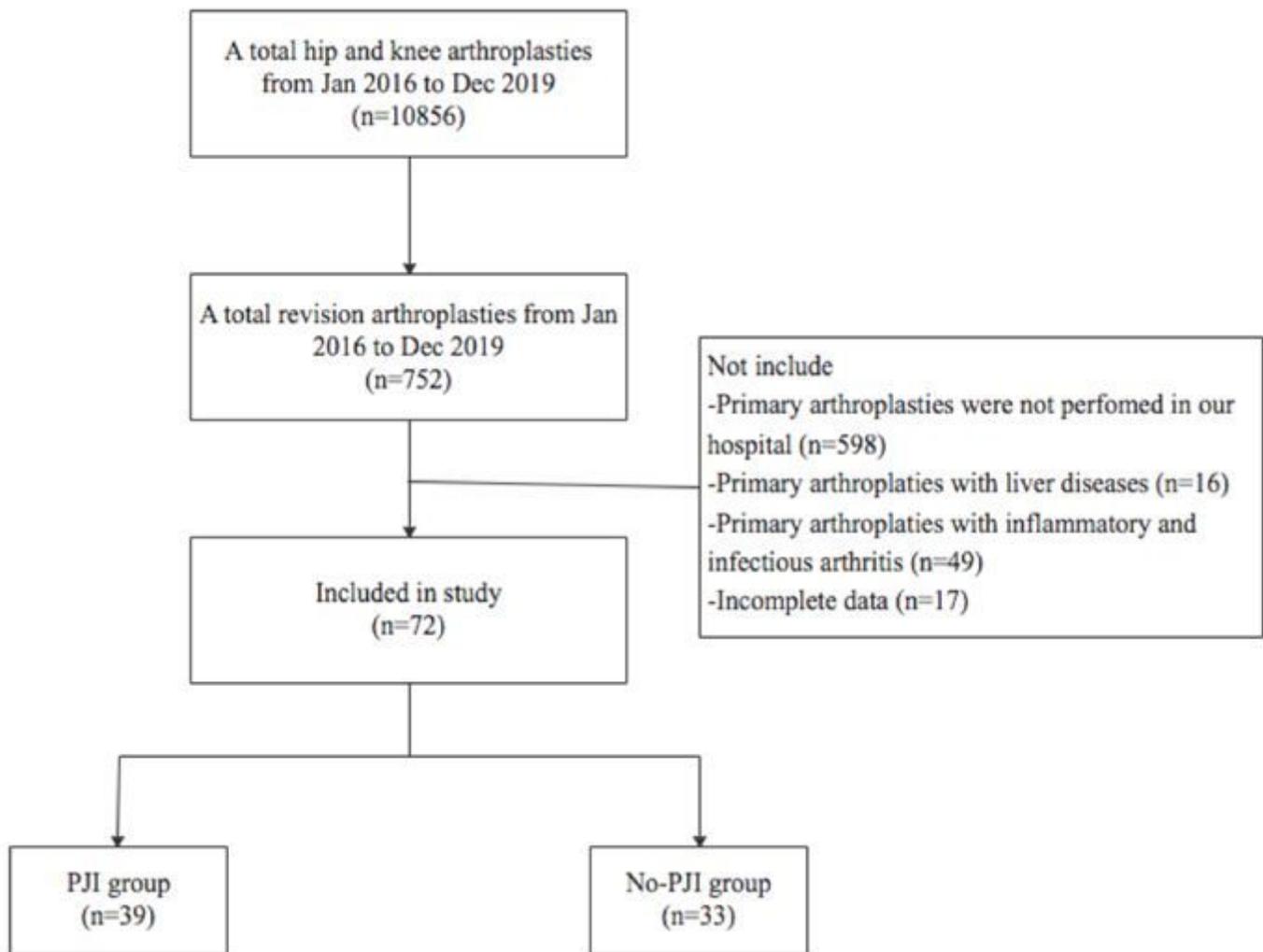
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## Figures



**Figure 1**

Patient inclusion and exclusion flowchart.



**Figure 1**

Patient inclusion and exclusion flowchart.

## ROC curve of total bilirubin

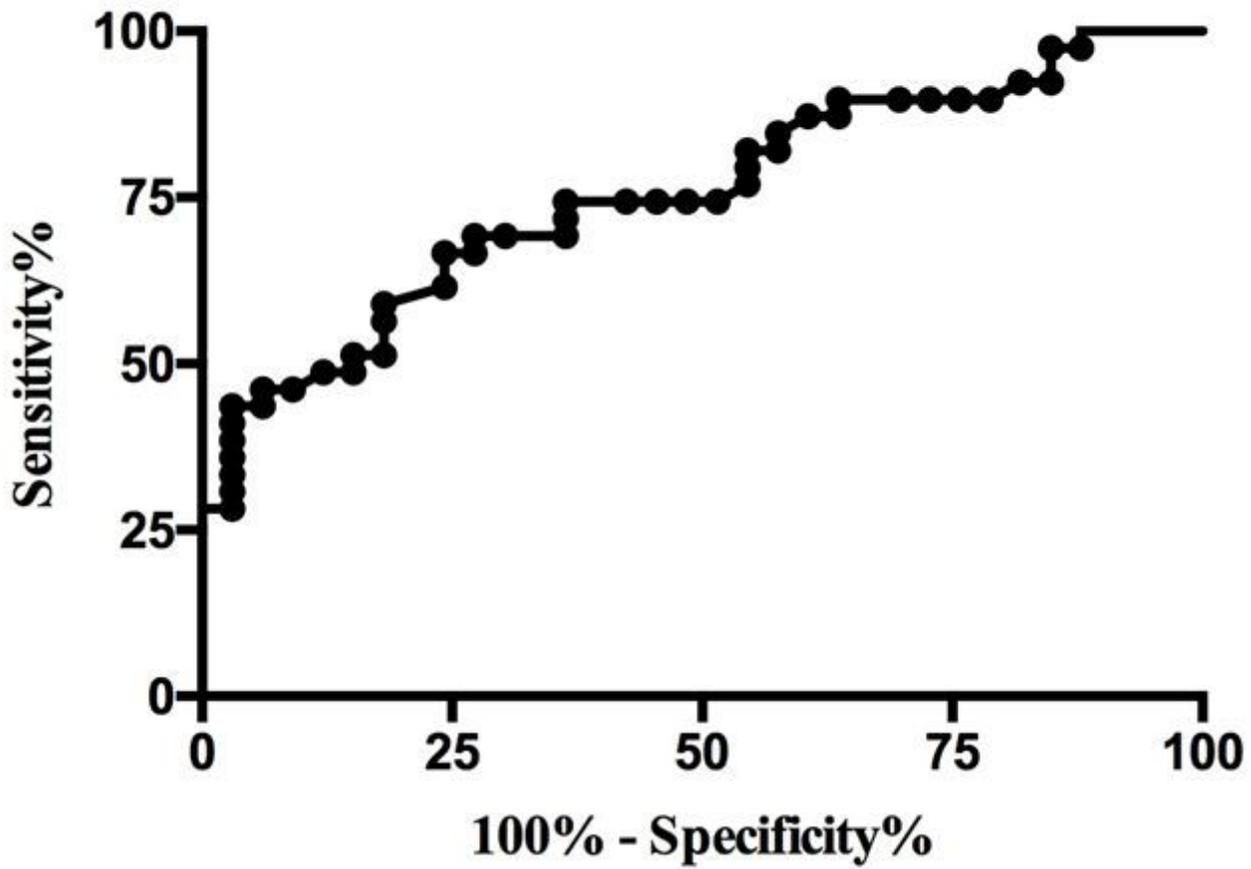


Figure 2

The ROC curve of total bilirubin to predict PJI. AUC=0.7552 (95%CI: 0.6448-0.8657,  $p=0.0002$ ), Cutoff=11.55  $\mu\text{mol/L}$ , Sensitivity=66.67%, Specificity=75.76%, PPV=76.47%, NPV=65.79%, OR=6.25 (95%CI: 2.21 - 17.65).

## ROC curve of total bilirubin

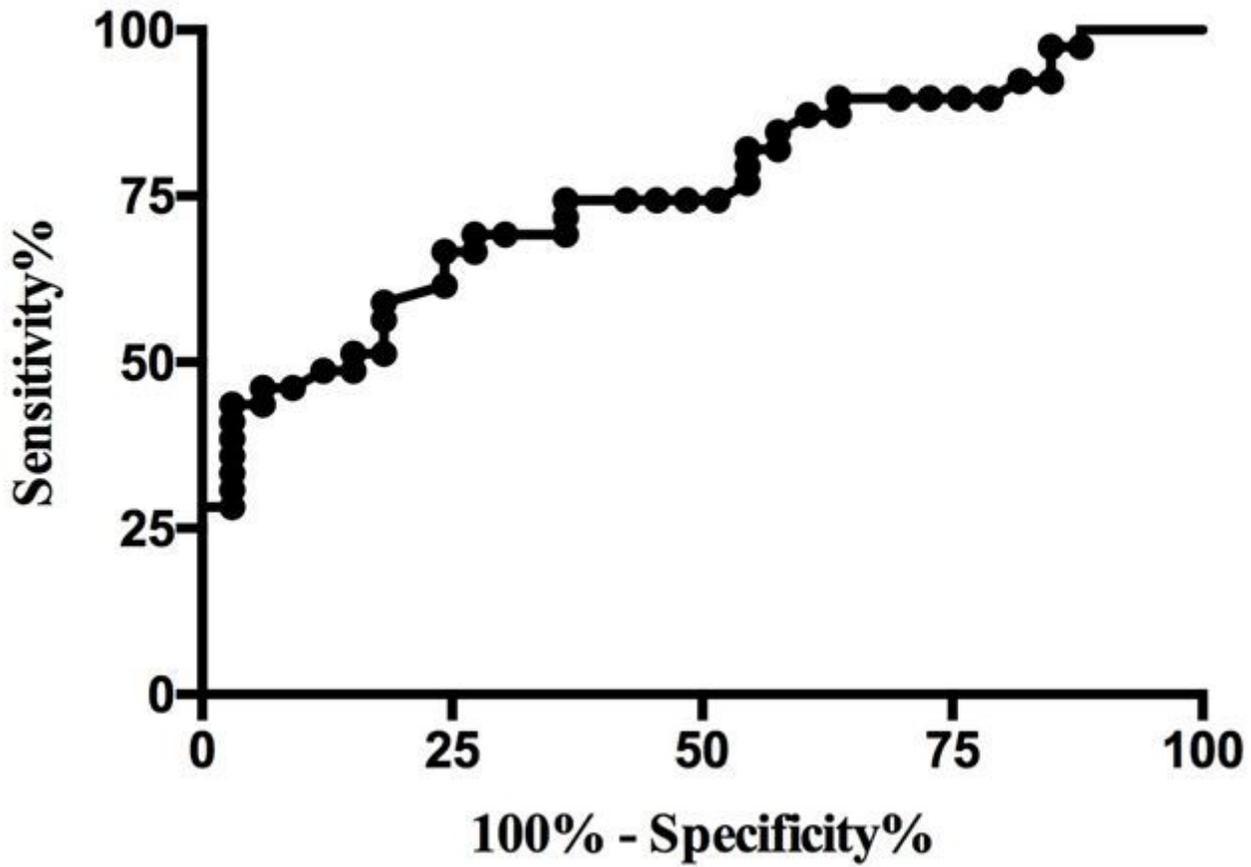


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## ROC curve of direct bilirubin

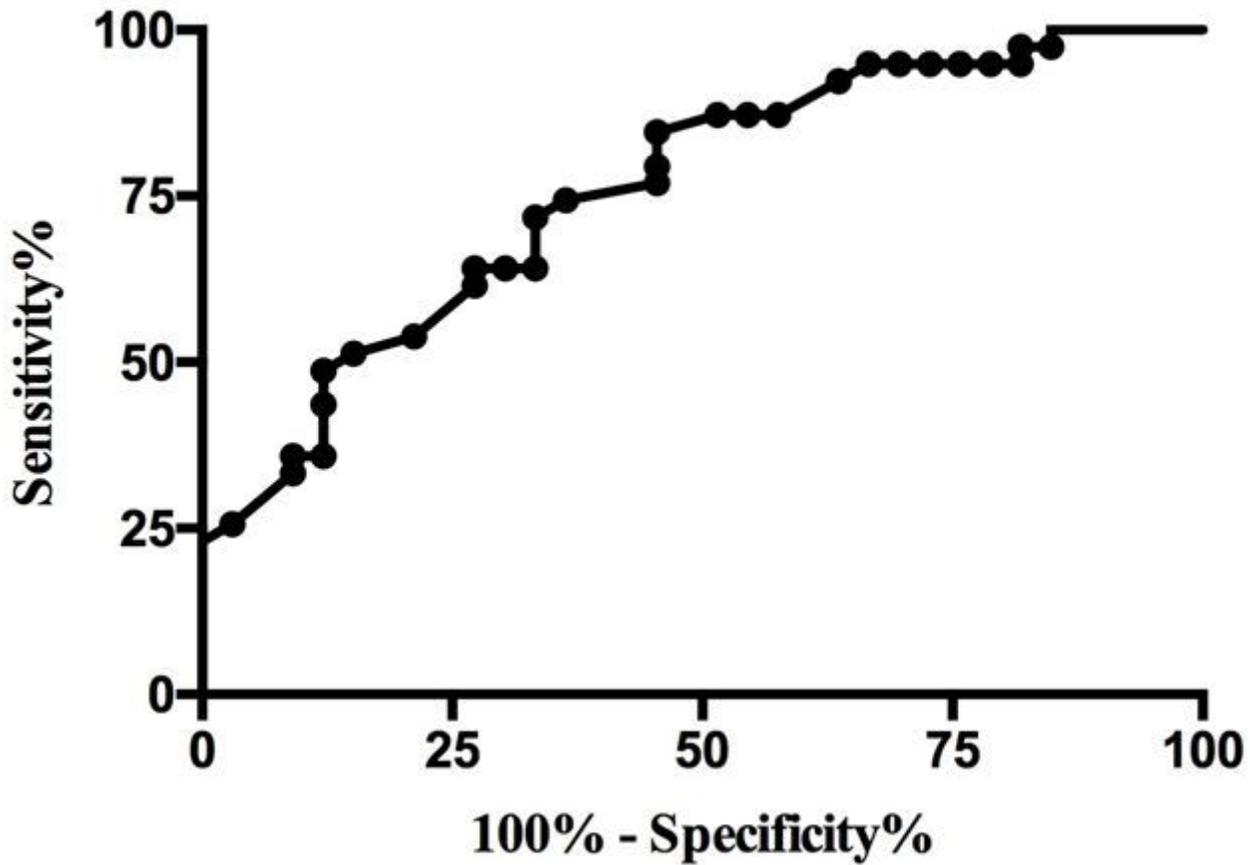


Figure 3

The ROC curve of direct bilirubin to predict PJI. AUC=0.7603 (95%CI: 0.6510-0.8696, p=0.0001), Cutoff=4.00 umol/L, Sensitivity=84.62%, Specificity=54.45%, PPV=68.75%, NPV=75%, OR=6.60 (95%CI: 2.18 - 19.98).

## ROC curve of direct bilirubin

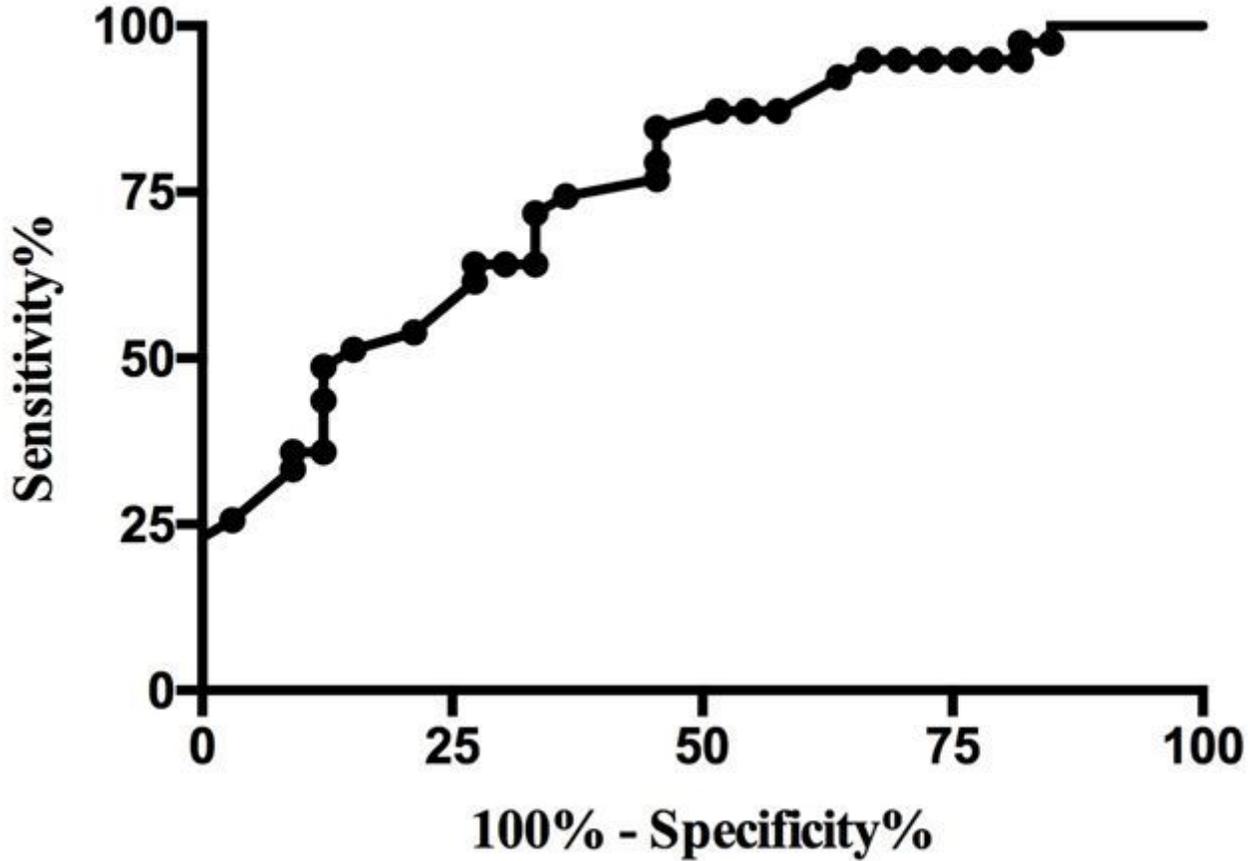


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## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

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