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Toll-Like Receptor 2 in Serum: a Potential Diagnostic Marker of Prosthetic Joint Infection?

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Prosthetic joint infection (PJI) is a severe complication of arthroplasty and is still lacking diagnostic gold standards. PJI patients display high Toll-like receptor 2 (TLR2) serum levels, correlating with canonical inflammatory markers (C-reactive protein [CRP], interleukin 6 [IL-6], tumor necrosis factor alpha [TNF-α], and IL-1). Therefore, TLR2 serum levels could be considered a new potential diagnostic tool in the early detection of PJI.

Toll-like receptor (TLR) represents the first line of defense against invading pathogens (1) by recognizing the invading bacteria and activating the inflammatory response aimed to eliminate the pathogen and repair the damaged tissue. Among TLRs, TLR4 and TLR2 recognize a broad spectrum of Gram-positive and Gram-negative bacteria, respectively, and induce the main inflammatory response (2, 3). Periprosthetic joint infection (PJI) (4–8) is one of the main adverse events of orthopedic surgical procedures (9–11). Currently, a large number of tests are available for PJI diagnosis, ranging from hematological markers of infection and inflammation to intraoperative culture and histology analysis. Nevertheless, there is still a lack of gold standards for the diagnosis of PJI (12, 13), because the clinical presentation of PJI is often ambiguous (14), and classical inflammatory markers can be misleading (15–17). In order to optimize the diagnostic process, infection biomarkers with fast response and high sensitivity and specificity for infection are needed (7).

In this context, TLR could be useful for PJI diagnosis. Indeed, TLR expression has already been considered of relevance in different inflammatory conditions and infections (18), but so far the potential diagnostic use of this molecule remains unexplored. For this reason, in this study the serum levels of the two main TLRs involved in bacterial infection, TLR2 and TLR4, were measured in PJI patients and in nonseptic patients undergoing implant revision and were compared to classical inflammatory parameters, such as C-reactive protein (CRP), and with the main inflammatory cytokines, interleukin 1 (IL-1), IL-6, and tumor necrosis factor alpha (TNF-α), in order to explore the potential use of TLR2 and TLR4 serum levels as novel diagnostic tools for PJI identification.

Patient population was described in Table 1. We selected 32 patients undergoing revision of total hip or total knee joint arthroplasty and displaying prosthetic chronic infection for at least 6 months, as demonstrated by clinical and laboratory signs typical of bone joint infection: swelling, erythema, joint pain, and secretion of purulent material. Diagnosis of infection was confirmed according to the criteria set forth by Spangehl et al. (19): at least three positive results for (i) erythrocyte sedimentation rate, (ii) C-reactive protein and aspiration, (iii) frozen section, or (iv) intraoperative culture. As a control, we selected 28 noninfected patients undergoing routine orthopedic surgery without any other underlying disease or infection of inflammation and showing no comorbid conditions that could affect the expression of TLR2 and other markers, no antibiotic therapy in progress, and no diabetes mellitus type 2 or obesity.

TABLE 1 Patient clinical features

Characteristic	Value	
	Controls	PJI patients
Amt of TLR (pg/ml) ± SD		
TLR4	348.81 ± 28.61	384.28 ± 43.84
TLR2	171.04 ± 34.36	504.19 ± 64.96
Amt of inflammatory marker (pg/ml) ± SD		
C-reactive protein	0.48 ± 0.27	2.63 ± 1.79
TNF-α	1.78 ± 0.51	6.70 ± 1.94
IL-1	2.19 ± 0.52	15.79 ± 3.62
IL-6	1.822 ± 0.75	11.2 ± 3.86
No. of patients with:		
Staphylococcus aureus (Gram positive)		11
Staphylococcus epidermis (Gram positive)		7
Staphylococcus xylosus (Gram positive)		1
Staphylococcus warneri (Gram positive)		1
Staphylococcus caprae (Gram positive)		1
Staphylococcus aureus (Gram positive)		1
Streptococcus anginosus (Gram positive)		1
Streptococcus agalactiae (Gram positive)		1
Enterococcus faecalis (Gram positive)		2
Corynebacterium striatum (Gram positive)		4
Klebsiella pneumonia (Gram negative)		1
Pasteurella multocida (Gram negative)		1
Staphylococcus aureus (Gram positive)/*Acinetobacter baumannii* (Gram negative)		1
Staphiylococcus aureus (Gram positive)/*Pseudomonas aeruginosa* (Gram negative)		1
No. of females and males	16 females, 12 males	16 females, 12 males
Mean age (yrs) ± SD	68 ± 22	63 ± 21

PJI and control patients were matched for age, sex, and severity

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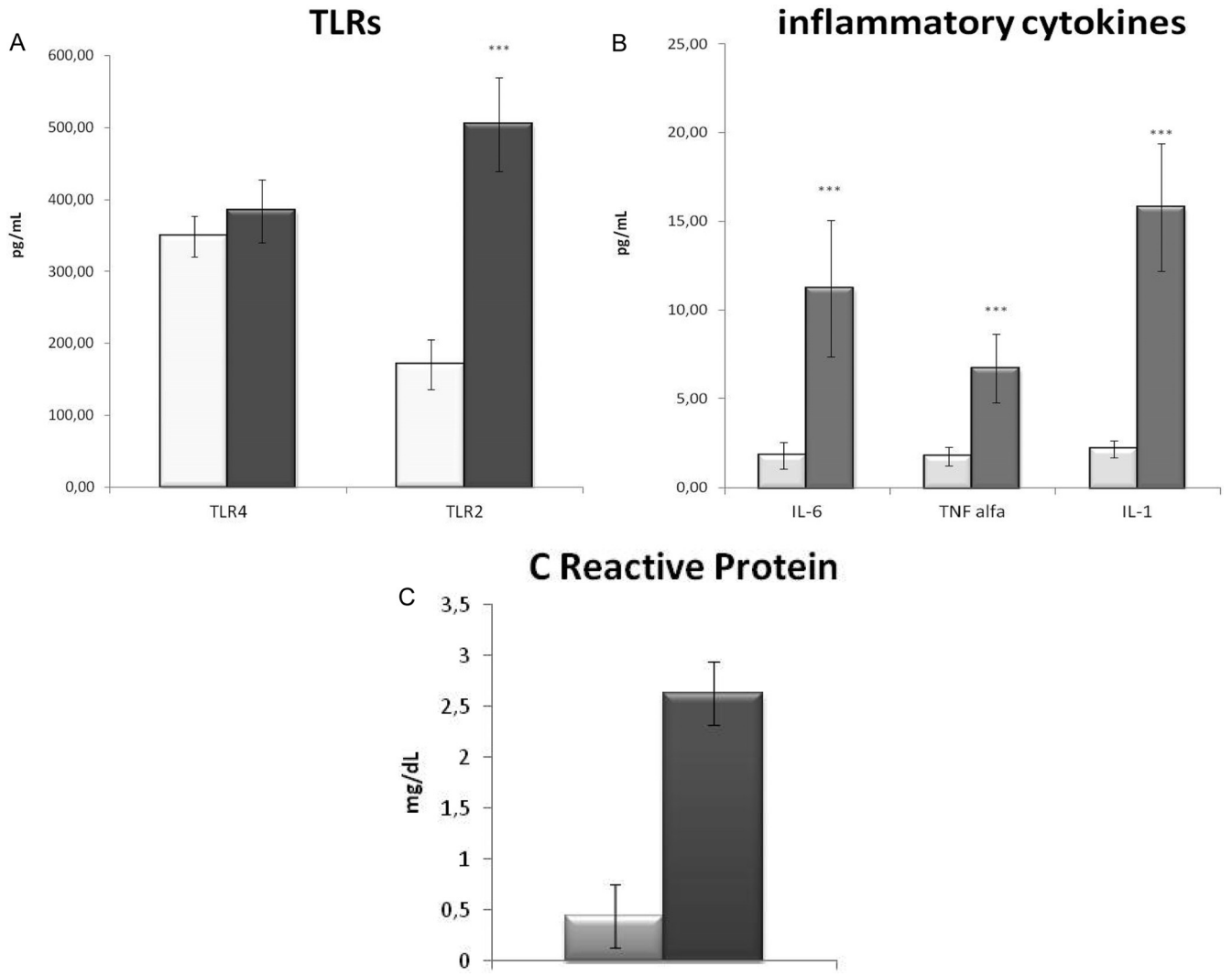

FIG 1 TLRs and inflammatory mediators. (A) Serum levels of TLR2 and TLR4 in prosthetic joint infection patients (gray bars) and noninfected patients (white bars); (B) serum levels of inflammatory cytokines (IL-1, IL-6, and TNF-α) in prosthetic joint infection patients (gray bars) and noninfected patients (white bars); (C) serum levels of C-reactive protein in prosthetic joint infection patients (gray bars) and noninfected patients (white bars).

of illness. Blood was drawn from all patients for serum separation, aliquoted, and stored at −80°C until further analysis.

CRP was measured using immunoturbidimetry on an automated biochemical analyzer (CRP-Latex assay; Olympus, Central Valley, PA, USA).

Human IL-1, IL-6, and TNF-α and TLR2 were measured in serum using an enzyme-linked immunosorbent assay (ELISA) sandwich duo set assay, according to the manufacturer's protocols (R&D Systems, Minneapolis, MN, USA). Human TLR4 serum concentration was measured by ELISA sandwich assay according to the manufacturer's protocols (USCN LifeScience Inc., Wuhan, Hubei, People's Republic of China; catalog number E90753Hu). TLR2 ELISA kit (DY2612; R&D Systems) was optimized for the analysis of TLR2 in cell supernatants and serum.

For all the parameters analyzed, normality of distribution of the three groups was verified by the Kolmogorov-Smirnov test for normal distribution.

Statistical analysis was performed using a one-way analysis of variance (ANOVA) test, and P values of <0.05 were considered significant and P values of <0.005 were considered very significant.

Linear regression analysis was performed between the different groups of data, and the 95% confidence interval of the regression line was calculated by using PRISM 3.0 software.

Surgical infection is due mainly to *Staphylococcus aureus* (20, 21), a Gram-positive bacterium recognized by TLR2 (22), while a small amount is due to Gram-negative bacteria, bound by TLR4. Accordingly, in infected patients, we observed 87.5% of Gram-positive and only 12.5% of Gram-negative infection and, as a consequence, a significant increase of TLR2 but not of TLR4 (Fig. 1). TLR2 has been described to be crucial in joint infection (20), contributing to the degenerative process and destructive arthropathy after microbial joint infection (23), indicating that TLR2 expression strictly reflects the progression of the infection in the host. So far, the alteration of TLR2 and TLR4 has been evaluated only at the gene expression level (24, 25), while the present work is the first, to our knowledge, which measures the amount of circulating protein, making it suitable for routine clinical diagnosis. In order to

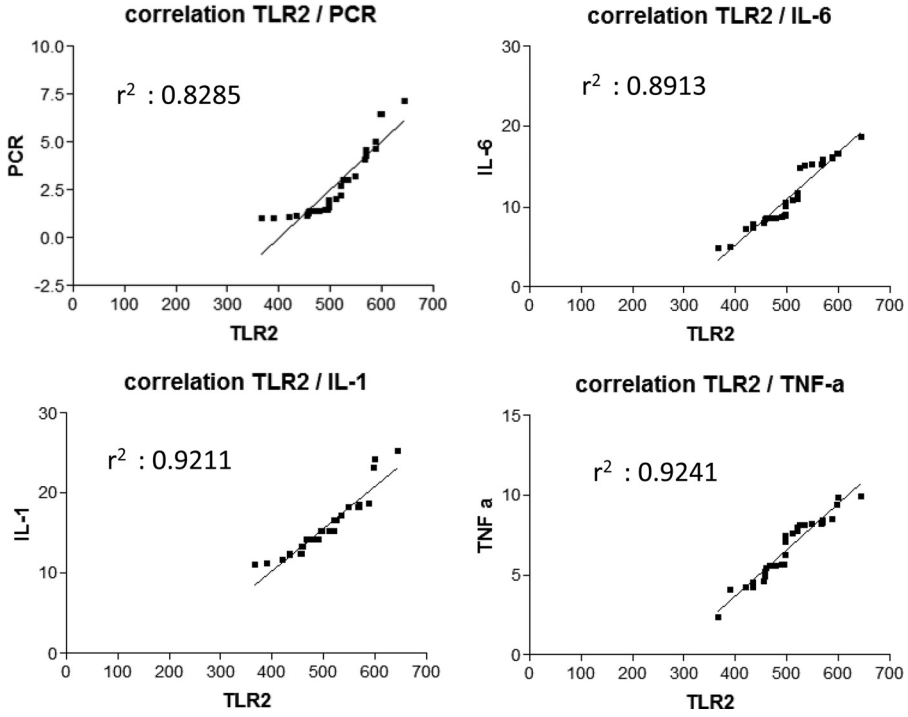

FIG 2 Correlation analysis. Linear regression analysis between TLR2 and inflammatory parameters. Top left, TLR2 and C-reactive protein (PCR); top right, TLR2 and IL-6; bottom left, TLR2 and IL-1; bottom right, TLR2 and TNF-α (TNF-a). For each analysis, coefficient of correlation r^2 is reported.

evaluate TLR2 circulating levels as diagnostic tools, we compared them with canonical markers of infection and inflammation. TLR2 showed a strong positive correlation with CRP (Fig. 1B), the gold standard clinical marker of infection, which is increased in PJI patients, indicating that the serum TLR2 molecule is able to detect an inflammatory condition. Moreover, since TLR2 mechanism of action leads to an inflammatory response (3), we measured the circulating levels of the three main inflammatory cytokines: IL-1β and TNF-α for local inflammatory response and IL-6 for systemic response. Infected patients displayed a significant increase of all the cytokines analyzed, in particular IL-6, previously described to be a significant marker of PJI (16), and IL-1β. In PJI patients, TLR2 displayed a strong positive correlation with both IL-6 and IL-1β, which exert a protective role on the tissue in *S. aureus* infection (26), confirming the importance of TLR2 in the detection of PJI (Fig. 2). Given the small sample size of patient groups, the results of this pilot study are preliminary, but taken together, they indicate that serum TLR2 can be considered, in association with canonical parameter of inflammation, a new potential diagnostic marker of PJI.

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