PROCALCITONIN, C-REACTIVE PROTEIN, INTERLEUKIN-6, AND SOLUBLE INTERCELLULAR ADHESION MOLECULE-1 AS MARKERS OF POSTOPERATIVE ORTHOPAEDIC JOINT PROSTHESIS INFECTIONS

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There is a universally recognized need to identify new, reliable markers of inflammation that can aid in the rapid diagnosis of orthopaedic joint prosthesis infections (OJP-Is). Since prompt diagnosis is key to timely intervention in the course of infection, different molecules have been studied. In this study, we examined three groups of patients: those with prosthesis infection, those without infection, and a third group with previous infection in whom the infection had been cleared. Four presumed markers of infection were tested: procalcitonin (PCT); C-reactive protein (CRP); interleukin-6 (IL-6); and soluble intercellular adhesion molecule-1 (sICAM-1). The results showed that PCT cannot be considered as a good marker of periprosthetic infection as no statistically significant difference in serum PCT levels emerged between patients with infection and controls or patients without infection. In contrast, both sICAM-1 and CRP may be considered as good markers of infection, as measurement of their levels allowed us to distinguish between patients with and without infection, and between patients with infection and those with previous infection, since marker levels quickly returned to baseline values after clearance of the infection. IL-6 was found to be a good marker for inflammation, as it distinguished between patients with infection and the other groups. In the patients with previous infection, the IL-6 values remained high versus the controls but lower and with a statistically significant difference versus the patients with infection. Further studies are needed to determine the cut-off value of IL-6 between patients with infection and those with previous infection.

A number of laboratory parameters can aid in the diagnosis of inflammatory disease and determination of immune response; however, few parameters are diagnostically useful for differentiating between acute bacterial infection and other types of inflammation. Most parameters in current use as indicators of inflammatory response (e.g., body temperature, white blood cell count, erythrocyte sedimentation rate) are aspecific for inflammation and tell us nothing about the cause underlying the inflammatory process (1). Postoperative orthopaedic joint prosthesis infections, whether after a first implant or a revision, are serious events requiring prompt diagnosis. The wide spectrum of clinical presentation and the differences in the efficacy of diagnostic approach continue to raise questions, and since microbiological determination is not always definitive, the search continues for alternative and/or

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complementary diagnostic methods.

Several recent studies on specific markers for prosthetic infection have investigated a variety of inflammatory molecules, including procalcitonin (PCT), C-reactive protein (CRP), and interleukin-6 (IL-6), all of which hold promise, although their potential utility remains controversial (2-3).

PCT, a protein consisting of 116 amino acids (molecular weight approximately 13 kDa), is an innovative diagnostic parameter and displays properties that differ from other currently available inflammatory markers. It is produced selectively in the presence of bacterial infection, sepsis, and the multiorgan dysfunction syndrome (MODS) (4).

In healthy people, plasma PCT levels are very low (50 pg/ml), while they may rise up to 1000 ng/ml in severe bacterial infections, without significant changes in plasma calcitonin concentrations. *In vivo*, PCT has a half-life of 25-30 h, which makes it useful as a diagnostic aid in daily practice. Recently, the hypothesis has been advanced that this prohormone is significantly synthesized also in the presence of local infection (2).

C-reactive protein, an alpha-globulin synthesized by the liver, dramatically increases its plasma concentration during acute inflammatory response (1, 5), reaching levels hundreds of times over baseline values within several hours, from 5-6 mg/l (normal range) to 500-1000 mg/l during inflammation (6). The rise in CRP is correlated with plasma IL-6 concentration (7), which stimulates adipocytes and hepatocytes to produce it. CRP levels are significantly increased in response to a variety of stimuli, including bacterial and viral infection. Elevated postoperative CRP values can persist up to 72 h, particularly in local prosthetic infection. A recent study demonstrated elevated CRP levels in 95% of patients with periprosthetic infection (8).

A member of the cytokine family, IL-6, an alpha-helical protein that undergoes phosphorylation and glycosilation (6, 9), plays an important role in inflammatory processes, acute phase response, bone metabolism and cancerogenesis. It is produced by many types of cells, including CD8+ T lymphocytes, fibroblasts, monocytes, osteoblasts, megakariocytes and adipocytes (10-13). Recent studies have found increased levels during the inflammatory phase in 95% of patients with local infection.

ICAM-1, a nearly ubiquitous membrane glycoprotein, plays a key role in leukocyte migration and activation (14-15). Its soluble forms (sICAM-1), both monomeric and dimeric, are generated by proteolytic cleavage (16-19). The majority of molecules that bind ICAM-1 are leukocyte integrins (LFA-1 and Mac-) (20-23), but also other types of molecules, including fibrinogen, rhinoviruses, and erythrocytes infected by *Plasmodium falciparum* can bind this molecule (24-25).

ICAM-1 is also involved in angiogenesis, wound healing and bone metabolism (26-28). Elevated sICAM-1 levels in serum, cephalorachidian liquid and bronchoalveolar lavage are associated with inflammation (15, 29-33).

In this study we analyzed blood concentrations of four inflammatory markers (PCT, CRP, IL-6, and sICAM-1) to determine their role in patients with acute orthopedic prosthetic infection, with previous orthopedic prosthetic infection, and in those undergoing implant revision without presenting signs of infection.

MATERIALS AND METHODS

The study population was 52 patients (24 males and 28 females) undergoing revision of total hip or total knee joint arthroplasty and subdivided into three groups according to the cause of prosthesis implant failure: 20 patients (6 males and 14 females) were categorized as having infection (Group A); 20 (16 males and 4 females) as having had previous infection (Group B); and the remaining 12 (Group C) as not having infection (2 males and 10 females).

Group A showed clinical and laboratory signs typical of bone joint infection: swelling, erythema, joint pain and secretion of purulent material, positive cultures (78% of cases) with isolation of the causal agent in the infectious focus. *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Staphylococcus epidermidis* were the most frequently isolated microorganisms. Antibiotic therapy consisted of fluoroquinolones, carbapenems, third generation cephalosporins or glycopeptide, depending on the susceptibility pattern of the causative microorganism.

The group with previous infection (Group B) was composed of patients in whom infection had been determined, had undergone surgical clearance of the infection site, and several months later (4-6 months) had undergone two-stage reimplantation. None of the patients in this group presented with clinical signs or symptoms of infection at the time of enrollment in the study.

The patients without current or previous known infections at the site of the joint prostheses (Group C) composed the control group and underwent revision arthroplasty for aseptic loosening of the implant. This group served to determine whether the selected markers could discriminate for the presence of bacterial infection.

Forty-six samples from apparently healthy subjects were analyzed in order to determine the reference range concentration of each marker in a healthy population.

Blood samples were taken from all the patients for serum separation, aliquoted and stored at -80°C until further analysis. Blood concentrations of PCT, CRP, IL-6, and sICAM-1 were measured. To determine PCT concentration, a quantitative electrochemiluminescence assay was run on an automated immune analyzer (Elecsys BRAHMS PCT, Hennigsdorf/Berlin, Germany). CRP was measured using immunoturbidimetry on an automated biochemical analyzer (Olympus CRP-Latex assay, Central Valley, PA, USA); IL-6 and sICAM-1 concentrations were measured using an ELISA sandwich assay (Quantikine®, R&D Systems, Minneapolis, MN, USA). Erythrocyte sedimentation rate (ESR) and leukocyte total count were also recorded.

Groups compared were: A *vs* B, A *vs* C and B *vs* C. Data were analyzed using Student's *t* test and linear interpolation. A p value of less than 0.05 was considered as statistically significant.

RESULTS

The three groups did not significantly differ for ESR (39.0±29.8 mm/hr, 19±18 mm/hr, and 19±13 mm/hr in groups A, B and C, respectively) and for total leukocyte counts (7,800±2,300 /mm^3, 7,600 ± 2,600 /mm^3, and 5,900±1,200 /mm^3 in groups A, B and C, respectively). The test results of the study population (n=52) are illustrated in Fig. 1-6.

Fig. 1. shows that, although PCT levels were higher in patients with infection than in those without infection (0.30 ng/ml *vs* 0.20 ng/ml), no statistically significant differences in the plasma PCT concentrations between patient groups (A, B and C) were observed (P=0.06). Fig. 2 reports the CRP values, which demonstrated a difference between the patients of group A and B *vs* group C (P=0.0001 and P=0.002, respectively). In nearly all patients with infection (95%), serum CRP was markedly higher than the reference range (<0.5 mg/dl), whereas in the patients with previous infection, it remained below this limit. The mean CRP concentration in patients with infection was 1.897±1.371 mg/dl. Fig. 3 shows the serum IL-6 values, which were found to be elevated in the patients with infection (9.125±5.026 pg/ml) and in those with previous infection (4.939±3.064 pg/ml). Statistically significant differences emerged between patients with infection and those with previous infection (P=0.002) and between patients with previous infection and patients without infection (P=0.0003). The mean IL-6, as calculated from the pool of healthy patients, was 3.002±0.548 pg/ml (range: 2.8-3.2 pg/ml; 99% confidence interval).

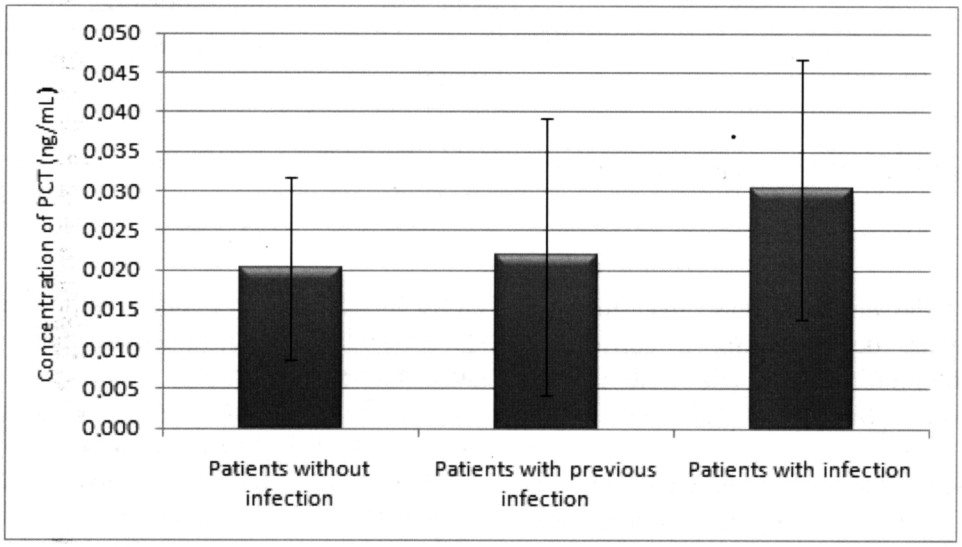

Fig. 1. *Serum procalcitonin levels.*

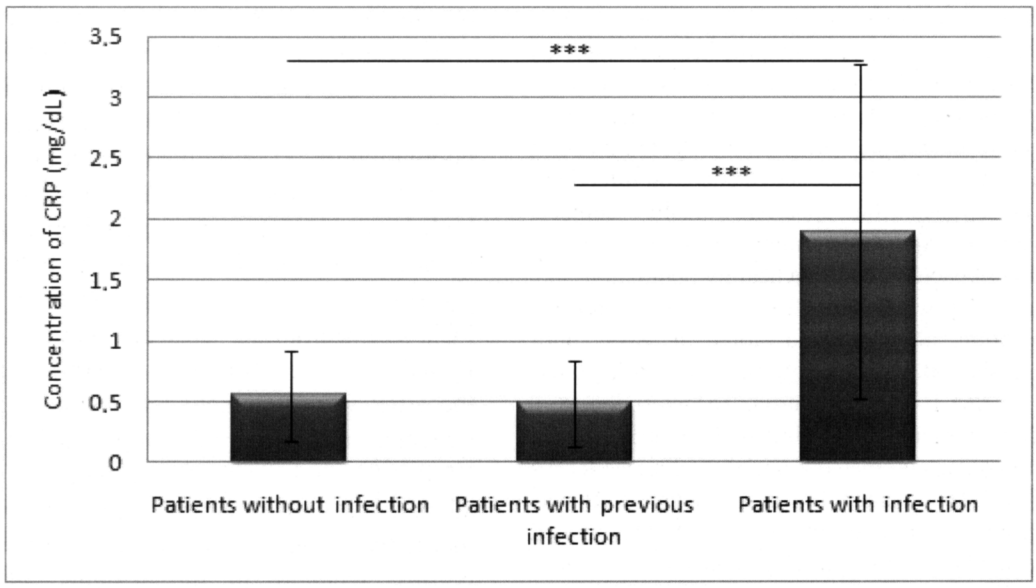

Fig. 2. *Serum C reactive protein (CRP) levels.* ***: *P<0.001.*

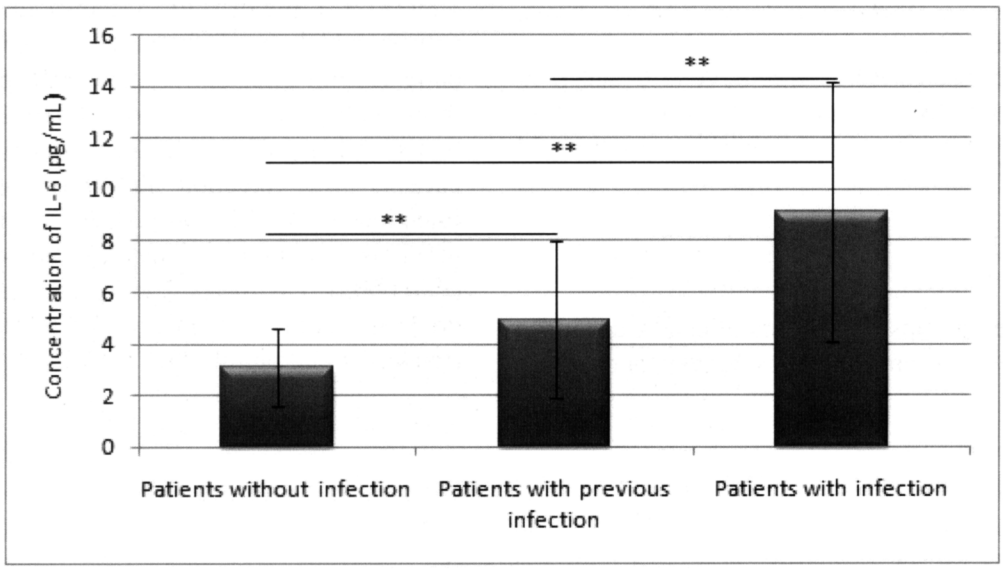

Fig. 3. *Serum interleukin-6 (IL-6) concentrations.* **: *P<0.02.*

Fig. 4 shows the linear decrease over time of serum IL-6 levels, demonstrating a correlation between concentration of IL-6 and time passed from the surgical clearance of infected tissues (R^2=0.678).

IL-6 values seemed to return to baseline values in about 6 months after the solving of infection, since no significant differences in IL-6 concentrations were found between patients without infection and patients with previous infection which had cleared at least 6 months before.

Fig. 5 reports the sICAM-1 concentrations and shows a statistically significant difference between patients with infection (Group A) and the other groups (P=0.01). In the patients with infection, the sICAM-1 mean concentration was 466.7±146.1

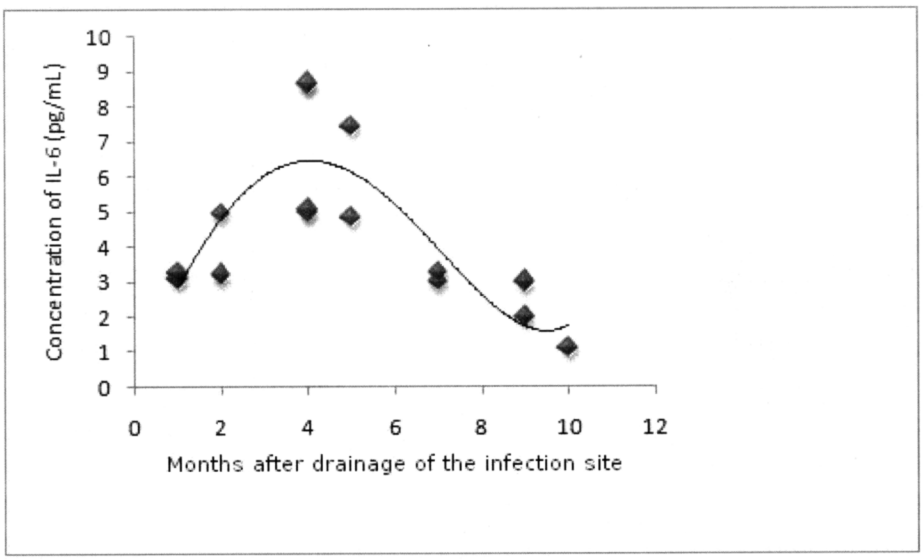

Fig. 4. *Serum interleukin-6 (IL-6) concentrations over time in patients of Group B. Polinomial IL-6 over time* R^2*= 0.678.*

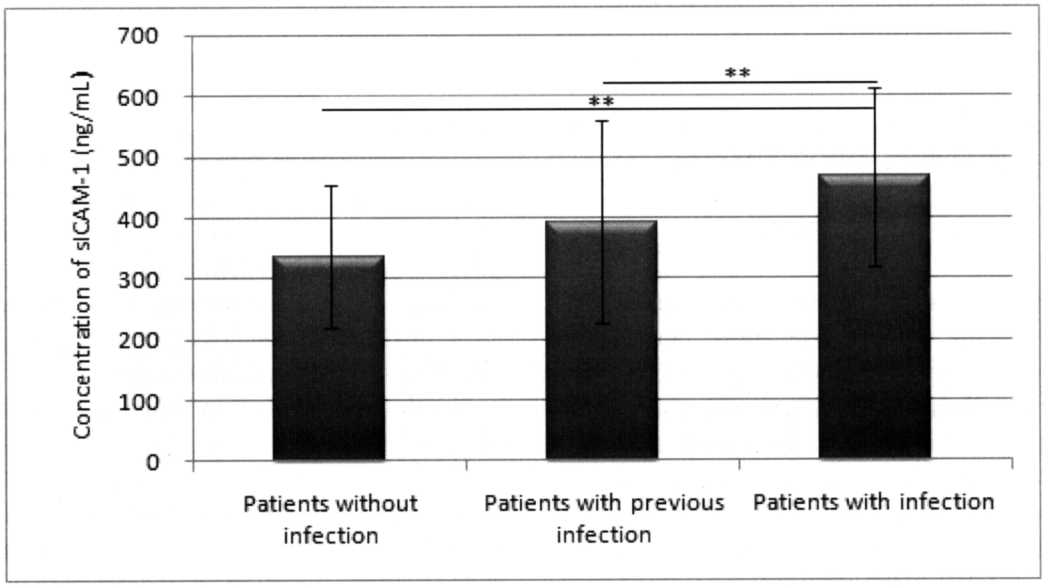

Fig. 5. *Serum soluble Intercellular Adhesion Molecule-1 (sICAM-1) concentrations. **: P<0.02.*

ng/ml, which was higher than the reference range (273.2-283.9 ng/ml; 99% confidence interval), as calculated from the pooled sample. The mean serum sICAM-1 levels were 396.6±165.9 ng/ml in the patients of group B and 336.6±116.1 in those of group C.

Fig. 6 shows the correlation between CRP, IL-6, and sICAM-1 in patients with infection. There was a linear proportionality between CRP and concentrations of the other parameters: R^2=0.91 between CRP and sICAM-1, R^2=0.86 between CRP and sICAM-1.

Therefore, CRP IL-6 and sICAM-1 were able to discriminate patients with a current infection from patients with previous or no infections. In addition, only IL-6 allowed to differentiate patients

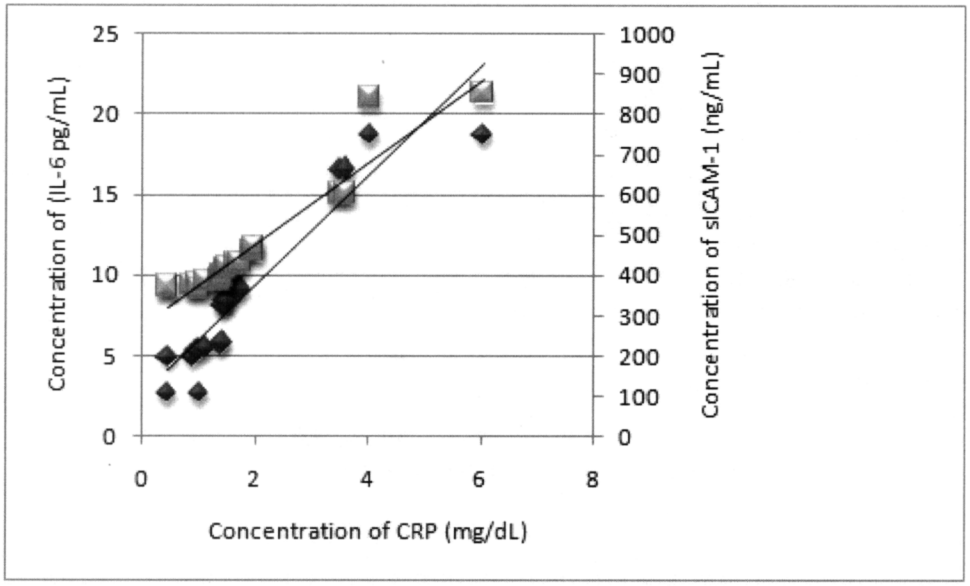

Fig. 6. *Correlation between C Reactive Protein (CRP), soluble Intercellular adhesion molecule-1 (sICAM-1) and Interleukin-6. Linear CRP vs IL-6 R2=0.8584. Linear CRP vs sICAM-1 R^2=0.9087.*

with previous infection from those with a current infection and from those with no infection.

DISCUSSION

Postoperative orthopaedic joint prosthesis infections remain one of the most challenging complications in patients undergoing arthroplasty. Early, accurate diagnosis of infection is essential for clinical outcome after surgery. Studies have focused on serum markers that can reliably and quickly demonstrate the presence of bacterial infection (34-35). One of the most widely investigated parameters is CRP. CRP may be considered as a good marker for inflammation, however, it is not sufficiently specific for establishing a bacterial cause of inflammation (1, 5-6). For example, elevated CRP levels may also be associated with trauma, viral infection or other causes. To aid in the diagnosis of infection, other markers besides CRP are needed, even if isolation of the pathogen whenever possible remains of paramount importance. Unfortunately, microbiological examination, the gold standard, may not always be feasible for a number of reasons such as prolonged antibiotic therapy, difficulties in collecting the optimal specimen or in culturing pathogens.

Recent research into infection markers has been extended to include other molecules such as PCT, IL-6, IgG, and sICAM-1 (11, 35-36), for some of which a reliable correlation with the infectious event has been demonstrated, while clinical value of PCT in diagnosis of post-operative joint prosthetic infections remains questionable (2, 4).

In this study on four candidate markers (PCT, IL-6, CRP, sICAM-1), we first categorized patients according to their clinical presentation. The study population was subdivided into patients with and without infection, plus a third group of patients with previous infection so to determine whether this borderline subgroup showed differences in marker levels. The patients were classified on the basis of findings from history taking, in which infection had been diagnosed and the pathogen(s) isolated from the periprosthetic material.

Despite the elevated specificity of PCT for systemic bacterial infection, PCT values were not useful for diagnosing periprosthetic infection. This observation contrasts with previous studies (2, 37) but agrees with a more recent research (38). On the other hand, measurement of CRP, sICAM-1 and IL-6 allowed us to distinguish between patients with

infection and the other two patient groups (those with or without a history of infection) and provides evidence that when combined these markers can aid in the diagnosis of local infection.

The use of IL-6 helped to distinguish between patients with infection and those without infection; however, other studies have indicated that IL-6 is not specific for detecting bacterial infection (18, 37, 39). IL-6 levels remained higher than the normal range also in the patients with previous infection, although to a lesser extent compared to patients with infection. In those with previous infection, IL-6 levels appeared to depend on the time from surgical clearance of the site of infection and its concentrations deceased more slowly than CRP ones. Unlike CRP, which returned to baseline values in patients with previous infection, IL-6 concentrations remained above the normal range over time, up to 6 months after clearance surgery. To the best of our knowledge, it is the first time that this result has been reported, even if further research is needed to set a reliable IL-6 cut-off value for discriminating between patients with infection and those with previous infection.

In conclusion, our data show that, besides isolation of the pathogen, the use of CRP,IL-6, and sICAM-1 as predictors of infection may help to establish a diagnosis of periprosthetic infection. Further study is needed to determine their real cut-off values and kinetics.

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