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Bacteraemia following periodontal procedures

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Abstract

Introduction: Transient bacteraemias are frequently detected following dental manipulation. Infective endocarditis (IE) can arise in susceptible individuals and antibiotic prophylaxis is routinely performed for certain procedures considered to be ''at risk'' of IE. Evidence is emerging that periodontal disease may be a significant risk factor for the development of certain systemic diseases such as cardiovascular disease. These systemic conditions could be initiated or detrimentally influenced by the repeated entry of bacteria into the bloodstream.

Materials and Methods: The present study comprised a single blind parallel study of 2 weeks duration. A baseline blood sample was obtained from 30 volunteers with untreated periodontal disease following which a periodontal probing depth chart was collected. A further blood sample was taken following this procedure, and each subject was recalled 2 weeks later. A blood sample was collected, the subject carried out toothbrushing and a further blood sample taken. Full-mouth ultrasonic scaling was then performed and a final blood sample taken. Blood samples were analysed for bacteraemia using conventional microbiological culture and polymerase chain reaction (PCR) using universal bacterial primers that target the 16S ribosomal RNA gene of the vast majority of bacteria.

Results: Using culture methods, the incidence of bacteraemias was as follows: following ultrasonic scaling (13%), periodontal probing (20%) and toothbrushing (3%). PCR analysis revealed bacteraemia incidences following ultrasonic scaling, periodontal probing and toothbrushing of 23%, 16% and 13%, respectively.

Conclusion: These findings suggest that detectable dental bacteraemias induced by periodontal procedures are at a lower level than previously reported.

Key words: bacteraemia; culture; periodontal disease; polymerase chain reaction

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Bacteraemia occurs when bacteria enter the bloodstream transiently and can be detected by laboratory blood culture techniques. There is evidence that dental procedures may cause bacteraemias in adults and that components of causative bacteria of oral infections, particularly lipopolysacharide, may promote atherosclerosis, affect blood coagulation, the function of platelets and prostaglandin synthesis (Syrjanen et al. 1989). Such functions are important in thrombus formation, which may lead to cerebral and myocardial infarction. Periodontal disease may thus contribute a significant risk for the development of systemic disease. In particular, cardiovascular disease may be detrimentally influenced by the repeated entry of bacteria into the bloodstream (DeStefano et al. 1993, Beck et al. 1996). Several accounts of transient bacteraemia in the literature have been directly related to dental disease and its treatment (Syrjanen et al. 1988, Christensen et al. 1993, Contreras & Slots 1996, van Winkelhoff & Slots 1999). However, it has been suggested that spontaneous bacteraemias associated with acute or chronic oral odontogenic infections, such as periodontal disease, may represent a far greater risk for the development of endocarditis than occasional health-care procedures administered in a professional setting (Murray & Moosnick 1941, Hockett et al. 1977, Guntheroth 1984, Bender & Barkman 1989, van der Meer et al. 1992, Durack 1995, Everett & Hirshman 1995).

Several studies have investigated the type and frequency of bacteraemias induced following different dental procedures and quote varying incidences (Bender et al. 1958, Rogosa et al. 1960, Rise et al. 1969, King et al. 1988, Heimdahl et al. 1990, Waki et al. 1990, Lucartorto et al. 1992, Hall et al. 1993, Okabe et al. 1995, Daly et al. 1997, da Fonseca 1998). Few of these studies are comparable because of differences in detection methods used, timing of blood sampling following the procedure, volumes of blood sampled and procedures and the clinical variables measured. Few studies have specifically investigated

bacteraemias associated with periodontal treatment. Furthermore, recently developed methods for sensitive and specific detection of microorganisms, particularly polymerase chain reaction (PCR), have brought renewed interest to this field.

The aim of this study was to assess patient- and practitioner-induced bacteraemia within periodontitis patients following routine periodontal procedures, namely periodontal probing, toothbrushing and ultrasonic scaling. The incidence of bacteraemia was assessed using conventional microbiological culture and PCR.

Material and methods

Subjects

Ethical approval for the study was obtained from the North Glasgow University Hospitals NHS Trust. Volunteers were invited to participate in the study after reading an information sheet and giving written informed consent. The study was designed and conducted according to guidelines for Good Clinical Practice.

The patients participating in the study had been referred to the Periodontology clinics at Glasgow Dental Hospital. Thirty-eight subjects were originally recruited but only 30 completed the study according to the protocol and thus eight were excluded. Patients who exhibited previously untreated, moderate-to-severe chronic adult periodontal disease, which was defined as having all quadrants with at least one pocket greater than 6 mm in depth, and who possessed a minimum of 20 teeth, were selected for inclusion in the study.

Exclusion criteria were subjects with abnormal haematological profile, those at risk of infective endocarditis (IE), subjects who had a significant medical or dental condition (such as diabetes, malignancy, or desquamative oral lesions, etc.), those taking any medication and those who gave a history of infectious disease (e.g. hepatitis, HIV), drug abuse or allergy to dental products or previous problems with venepuncture. Subjects who had incompatible dentition, e.g. orthodontic bands, partial dentures or teeth unsuitable for extensive ultrasonic scaling, were also excluded.

Clinical protocol

At the initial visit, subjects who wished to participate gave written informed consent, a detailed medical history and a baseline blood sample (sample 1). Following a general oral examination, a full-mouth periodontal probing depth chart was then taken using a Pocket Charting Probe (PCP) 12 periodontal probe. Periodontal probing depths, in addition to gingival recession and loss of attachment levels for six sites per tooth were recorded and a subject mean derived. Bleeding on probing (BOP) inter-proximally and mobility scores were recorded for each tooth. A blood sample was taken immediately (range 30 s to 1 min) following this procedure (sample 2). Any subject beginning a course of medication during the study was asked to inform the clinical assessor in order that their eligibility to continue in the study could be assessed.

At the second visit, a baseline blood sample was taken (sample 3). The patient performed supervised toothbrushing for 2 min and a further blood sample was then taken immediately after this (sample 4) (never more than 3 min). A full-mouth ultrasonic scaling was then performed and immediately following this, a final blood sample was taken (sample 5).

Blood sampling

Blood was obtained from veins in the antecubital fossa. Prior to each sampling, the site was wiped with isopropyl alcohol to minimize the number of potential skin contaminants. Each sample comprised 28 ml of blood, which was obtained using a 19-gauge butterfly and safety lock blood collection set, 20 ml syringe and vacutainer holder, which were all attached to a Connecta TH three-way stopcock (Ohmeda, Helsingborg, Sweden). Two 4.4 ml vacutainer tubes containing ethylenediaminetetraacetic acid (EDTA) (Becton Dickinson, Oxford, UK) were used for the collection of samples required for PCR analysis.

Inoculation of blood to Bactec bottles

Twenty millilitres of venous blood was used to inoculate two Bactec bottles (Organon Teknika, Cambridge, UK). Ten millilitres of blood was inoculated into a FAN aerobic (1041) and FAN anaerobic (1042) culture bottle using a Microlance 3 needle (Becton, Dickinson and Company, Franklin lakes, NJ, USA). The Bactec bottles were incubated and continuously monitored over 14 days for the presence of microorganisms. Samples that indicated positive for bacteria were then further analysed as detailed later.

Microbiological culture and bacterial identification

Samples from culture-positive bottles were inoculated by means of sub-venting culture units (Organon Teknika) onto two Fastidious Anaerobe Agar (FAA) plates (Bioconnections, Leeds, UK) supplemented with 7.5% v/v sterile defibrinated horse blood, and also onto two Columbia agar plates (Bioconnections) supplemented with 5% v/v sterile defibrinated horse blood. The FAA plates were incubated in an anaerobic chamber (Don Whitley Scientific, Shipley, UK) at 37°C and two Columbia Blood Agar (CBA) plates were incubated in an atmosphere of 5% CO_2/85% air at 37°C. All plates were incubated for up to 14 days and examined daily for the presence of bacterial growth.

Preliminary identification of pure bacterial cultures was based on aerotolerance, colony and cellular morphology and Gram staining characteristics. Biochemical tests were performed using commercially available diagnostic kits designed for bacterial identification. Anaerobic isolates were identified by means of the Rapid ID 32A kit (Biomerieux, Basingstoke, UK) using 29 standardized enzymatic and biochemical tests. Identification of *Streptococcus* species was accomplished using the API 32 STREP kit (BioMerieux). Tests were performed, and the results obtained and interpreted in accordance with the manufacturer's instructions.

PCR

Chromosomal DNA was extracted from whole blood using the QIAamp™ DNA blood mini kit (Qiagen Ltd., Crawley, UK) in accordance with the manufacturer's instructions. Universal bacterial primers that targeted a conserved region of the 16S ribosomal RNA of the vast majority of bacteria were used in the PCR assay. Primer sequences were 5′-AGA GTT TGA TC(AC) TGG CTC AG-3′ (27f) and 5′-TAC GG(CT) TAC CTT GTT ACG ACT T-3′ (1492r). This primer pair yields a 1505-bp PCR product.

PCR amplification was carried out in a reaction volume of 50 µl. Each PCR reaction mixture comprised 5 µl of sample and 45 µl of reaction mixture containing 1 × PCR buffer (10 mM Tris-HCl pH 8.8, 1.5 mM $MgCl_2$, 50 mM KCl, 0.1%

Triton X-100), 1.0 U of *Taq* DNA polymerase (Amersham Pharmacia Biotech, Milton Keynes, UK), 0.2 mM dNTPs and each primer at a concentration of 0.2 μM. The specificity and yield of reaction products was maximized by using ''hot start'' PCR, in which the primers were separated from other components of the reaction mixture by a layer of wax (Flowgen, Lichfield, UK), and thereby preventing the reaction from starting until the wax had melted upon the commencement of thermal cycling. PCR cycling conditions comprised an initial denaturation step of 94°C for 5 min, followed by 35 cycles of denaturation of 94°C for 1 min, primer annealing at 55°C for 1 min and extension at 72°C for 1.5 min, and then a final extension step at 72°C for 10 min. Two rounds of PCR were performed, using 5 μl of the first round PCR product as a template for the second round of amplification.

Stringent anti-contamination procedures were employed when carrying out PCR. Separate areas were used for sample preparation, setting up of PCR reactions and post-PCR analysis of reaction products. Filter tips were used at all stages when setting up PCR reactions and samples were added to reaction mixtures with a positive displacement pipette. Positive and negative reaction controls were included with each batch of samples being analysed. The positive control used was a standard PCR reaction mixture containing 10 ng of *Prevotella intermedia* genomic DNA instead of sample, whereas the negative control contained sterile water instead of sample.

Fifteen microlitres of each reaction product was added to 2 μl of gel loading dye (0.25% bromophenol blue, 50% glycerol, 100 mM EDTA pH 8.0) electrophoresed on a 2% agarose gel containing ethidium bromide (0.5 μg/ml) and visualized on a UV transilluminator. A 100-bp DNA ladder (Amersham Pharmacia Biotech) was used as a size marker to assist the analysis of the PCR products.

Results

Microbiological culture

Thirty-eight subjects were recruited into the study but eight did not comply with the protocol and were withdrawn from the study. Of the 30 participants, 18 were male and 12 were female with a mean age of 42.3 years (range 24.8–64.6 years). Baseline samples from two subjects were positive for bacteria using the Bactec system, but on subsequent subculture no bacteria could be isolated (Table 1) possibly because of incompatibility of the culture plates for those particular organisms. In all tables, results are scored as presence of positive culture or PCR-positive results regardless of whether the baseline was positive or negative. Following periodontal probing, samples from six subjects were positive using the culture system. However, one of these samples provided no bacterial growth on subculture. Subsequent subculture of the remaining five samples resulted in the isolation of several bacterial species. From subject 1, *Propionibacterium acnes*, *Neisseria pharyngis*, *Streptococcus viridans*, *Micrococcus* spp. and *Staphylococcus albus* were isolated. *Anaerobic streptococci* and *Micrococcus* spp. were isolated from subjects 8 and 14, respectively. Subject 26 yielded *P. intermedia* and *Actinomyces naeslundii*, while *Haemophilus aphrophilus* and coagulase-negative *Staphylococcus* species were isolated from subject 35. Following toothbrushing, one subject provided a positive blood culture by Bactec and on subculture the isolate was identified as *Gemella haemolysans*. Following ultrasonic scaling, four bottles from three subjects were positive by Bactec. *A. naeslundii* was isolated from subject 1. Subject 13 yielded *Streptococcus parasanguis*, *A. naeslundii*, *Eubacterium* spp. and *Eubacterium limosum*. *P. acnes* was the only bacterium isolated from subject 19.

Table 1. Positive microbiology culture results for all samples taken at each time point

Subject	Sample	Growth conditions	Results of subsequent API identification procedures
IR004	Baseline	AnO_2	No growth
JM001	Baseline	AnO_2	No growth
IL008	After probing	AnO_2	*Anaerobic Streptococci*
IR004	After probing	AnO_2	No growth
JM001	After probing	AnO_2	*Propionobacterium acnes*, *Neisseria pharyngis*, *Streptococcus viridans*, *Micrococcus*, *Staphylococcus albus*
CG035	After probing	O_2	*Hemophilus aphrophilus*, Coag-ve *Staphylococci*
FJH014	After probing	O_2	*Micrococcus*
MM026	After probing	O_2	*Prevotella intermedia*, *Actinomyces naeslundii*
ND021	After brushing	O_2	*Gamella haemolysans*
JK013	After scaling	AnO_2	*Streptococcus parasanguis*, *A. naeslundii*, *Eubacterium* sp., *Eubacterium limosum*
JM001	After scaling	AnO_2	*A. naeslundii*
JK013	After scaling	O_2	*Streptococcus parasanguis*
LM019	After scaling	O_2	*Propionobacterium acnes*

All positive Bactec results are shown and all subsequent culture results even if no identification was made by the API system. Where several organisms were detected these are named.

Table 2. Positive polymerase chain reaction analyses from all subjects and blood sampling time points

Subject	Sample
TMcQ002	Baseline
MS007	Baseline
EM010	Baseline
JS005	After probing
CJ038	After probing
MS007	After probing
LL016	After probing
EM010	After probing
JS024	Second baseline
AH031	After brushing
RS003	After brushing
DR027	After brushing
FM009	After brushing
FJH014	After scaling
IR004	After scaling
JK013	After scaling
DR027	After scaling
SM029	After scaling
CJ038	After scaling
AD028	After scaling

PCR

PCR was performed on chromosomal DNA extracted from whole blood. Since all samples were PCR negative following a single round of PCR, the sensitivity of PCR was increased by carrying out a second round of PCR. This was indicative of the low levels of bacteria in the samples analysed. Several samples were PCR positive following the second round of amplification (Table 2). A representative result from the agarose gel electrophoresis of PCR products from blood samples is shown in Fig. 1.

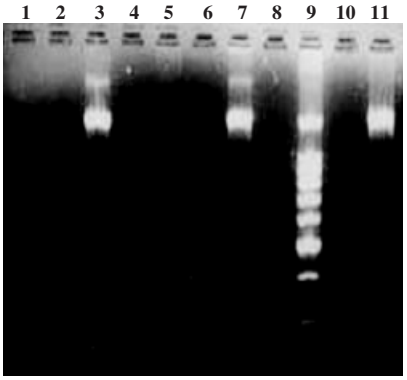

Fig. 1. Electrophoresis on 2% agarose gel of selected polymerase chain reaction products obtained from blood samples using the bacterial 16S rRNA primers 27f and 1492r. Lanes: 1–8, blood samples; 9, 100-bp DNA ladder; 10, negative control; 11, positive control.

Table 3. Culture and PCR results by event with the percentage of bacteraemia positives in parentheses

	Baseline	After probing	Second baseline	After brushing	After scaling
Culture	2 (6%)	6 (20%)	0 (0%)	1 (3%)	4 (13%)
PCR	3 (9%)	5 (16%)	1 (3%)	4 (13%)	7 (23%)

PCR, polymerase chain reaction.

Table 4. PCR-positive results grouped according to the nearest mean subject pocket depth

Mean pocket depth (mm)	*n*	Baseline	Probing	Second visit	Toothbrushing	U/S scaling
2	1	0	0	0	0	0
3	13	2 (14.3%)	1 (7.7%)	1 (7.7%)	1 (7.7%)	2 (15.4%)
4	11	1 (9.1%)	3 (27.3%)	0	3 (27.3%)	3 (27.3%)
5	4	0	0	0	0	0
6	1	0	0	0	0	1 (100%)

PCR, polymerase chain reaction; U/S, ultrasonic.

At baseline during visit 1, three samples were PCR positive and one sample was PCR positive at baseline visit 2. Following periodontal probing, five samples were PCR positive. Four samples were PCR positive following toothbrushing. Seven samples were PCR positive following ultrasonic scaling. Table 3 shows the comparison of both the blood culture and PCR results by event and facilitates comparisons.

Table 4 shows the number of PCR-positive results by event and grouped according to the nearest mean subject pocket depth. The PCR data, but not the culture data, show a trend for more positive samples in the deeper pocket depths but this was not statistically significant because of the low number of subjects. Less conclusive data were available for culture results by pocket depth (data not shown). Similar inconclusive results occurred for PCR and culture detection when compared with BOP. BOP was relatively high in these subjects, in that 20 subjects had BOP> 50% and 10 were below this level. The incidences both conventional culture and PCR of bacteraemias were split proportionally between these groups (approximately 50:50) suggesting a limited influence of bleeding tendencies in these patients on bacteraemias.

Discussion

We nominally state that bacteraemias are present when either PCR or culture methods detect a positive sample, which is consistent with the current literature (Peters et al. 2004), although strictly speaking the term ''bacteraemia'' refers to live organisms in the bloodstream and PCR does not discriminate live and dead bacteria. Never-the-less the PCR technique is very sensitive and we sought to determine the most efficacious method between the two techniques and to utilize the perceived greater sensitivity for small amounts of bacteria that PCR permits. The Bactec method does not compare in sensitivity to optimized culture plates and conditions for specific bacteria but our rationale for using this system was that this was the standard in medical bacteraemia research of blood samples.

The results of this study suggest that periodontal probing, toothbrushing and ultrasonic scaling can produce a detectable bacteraemia. A number of factors may play a role in the ability to detect a transient bacteraemia. These include the timing of the blood sampling (Schotmuller 1925, Cobe 1954, Elliott & Dunbar 1968, Baltch et al. 1982, Berger et al. 1988, Hall et al. 1996, Lockhart 1996, Roberts 1999), techniques used for bacteraemia detection such as bacterial culture (Coulter et al. 1990, Heimdahl et al. 1990), and may include the degree of periodontal inflammation, and quantity and composition of the gingival flora.

The subjects in this study were ''untreated periodontitis'' patients and it was expected that a high incidence of bacteraemia following periodontal procedures would be detected, and that the isolates would reflect the complex anaerobic flora found in periodontal pockets. We found it impossible to predict which subjects would experience a detectable bacteraemia as there was no clear association between severity of disease and detection of a bacteraemia (Table 4). Subjects with the deepest pocketing were not necessarily those in whom bacteraemia was detected, indicating that more healthy patients may be at risk of physiological bacteraemia or that periodontitis patients may have processes developed to minimize bacteraemias, irrespective of periodontal pocket depth (Table 4). Ultrasonic scaling was the procedure that induced bacteraemia on most occasions, which can be appreciated given the high tissue trauma induced by mechanical instrumentation and water spray. Periodontal probing also resulted in a high incidence of bacteraemia. This supports the theory that bacteria are readily carried into crevicular and subgingival tissues with this procedure. Our values were lower than those quoted by Berger et al. (1988) following dental extractions, which supports the idea that less trauma results in lower bacteraemia levels. The bacteraemia levels found in our study are lower than that quoted by other workers using similar periodontal procedures. Waki et al. (1990) detected bacteraemia in 18.5% of samples following scaling. Interestingly, Bender & Barkman (1989) detected a bacteraemia rate of 30% immediately following scaling which reduced to 5% ten minutes later. Our lower values taken immediately following the procedure are similar to the finding of Bender & Barkman (1989) at 10 min. In addition, as highlighted by other workers (Roberts et al. 1997), there may have been too long a gap

between cessation of a manipulative procedure, application of a tourniquet and blood sampling time. Our finding may be unique to periodontal patients whose immune systems readily and regularly have to deal with bacteraemia associated with periodontal pocketing. The relatively low incidence of bacteraemia in the present study may be a result of our conventional microbiological detection methods being of insufficient sensitivity.

Occasionally, Bactec bottles that were culture positive provided no growth on subsequent subculture. One explanation could be that the bacteria in these cases were uncultivable on CBA and FAA, although they may have proved positive by PCR. Alternatively, high levels of white blood cells in the blood sample may have given a false-positive result with the Bactec culture bottles. Since many dental bacteria are particularly slow growing, the relatively long incubation time of 14 days, compared with the 5–7 days of incubation performed in other bacteraemia research, may have led to several more bottles becoming culture positive (Kara et al. 2004). Both culture media used in this study sustained growth and met the atmospheric requirements of all species tested by the manufacturers. However, it is known that several species of bacteria, such as *Campylobacter*, will not be supported by the Bactec medium (Hutchinson et al. 1992) and it is therefore likely that particularly fastidious dental isolates may not have been sustained. PCR could be beneficial in the detection of such species. The results obtained show that a greater number of samples were positive by PCR compared with conventional microbiology, indicating the greater sensitivity of the PCR method. Indeed, four samples were positive at baseline, supporting the theory of low-level transient physiological bacteraemia without manipulation (Ley et al. 1998).

Samples that were positive by PCR were not necessarily the same samples as those that had been positive by conventional microbiology. Indeed, only two samples were positive by both PCR and culture. Interestingly, Muller-Premru & Cernelc (2004) found similar inconsistent results but in paired blood samples, the results did not agree in 30% of cases (four out of 14). It should be borne in mind that the actual blood aliquots tested by PCR and conventional microbiology were not the same. It was not technically feasible to use the contents of the Bactec bottles as the PCR sample, since an inhibitor in the Bactec bottles prevented PCR being performed. In addition, several organisms may be uncultivable and therefore only detectable by PCR. Indeed, 16 non-baseline samples were positive by PCR alone, compared with 10 blood culture samples that provided an identifiable organism with conventional microbiology on subculture.

Despite the 27f/1492r PCR primer pair used being able to detect most bacterial species, it has been noted in other studies that *Actinomyces* species are detected relatively infrequently with these primers. This could explain why three of the samples culture positive for *Actinomyces* species were negative by PCR. Several samples were positive by PCR but negative by culture. This could have been because of several reasons. For example, some of the positives detected by PCR may be non-fermentive and the BacT/Alert system detects organisms by the colorimetric detection of CO_2 produced by growing organisms. It is possible that the number of positive blood culture samples is an underestimate, and some of the bacteria in the bottles that were found to be positive by Bactec but provided no subsequent growth on subculture may simply have exhausted the nutrients in the medium and died before subculture was attempted. It should be highlighted that PCR will detect dead and moribund organisms that may be present in the bloodstream, which should result in more positive samples when tested by PCR than by conventional blood culture.

Because of the extreme sensitivity of the PCR technique, any contamination occurring during blood specimen collection (the skin during venepuncture), or in laboratory processing will lead to false positives (Victor et al. 1993). The most common problem is carryover of amplicons from previous reactions. However, contamination was not a problem in the currently reported study as the contamination protocols used were stringent. Samples positive by PCR but which were negative by culture may indicate background contamination from organisms obtained during specimen collection, lack of specificity of the primers used if looking for a specific organism (Greisman & Hornick 1969) or subclinical bacteraemia or DNAaemia that may represent killed fragments of bacteria in the bloodstream (Heininger et al. 1999). The use of reverse transcriptase PCR may be useful to identify bacteria that are actively replicating and thus clinically important.

Animal studies indicate that periodontal disease does increase the incidence of IE and that the number of microbes entering the bloodstream may not be as important in the production of IE as other qualities, such as the ability of the microbe to adhere (Glauser & Francioli 1987).

In conclusion, it is evident that bacteraemia does arise following various clinical periodontal manipulations (13–20%) as well as following toothbrushing (Syrjanen et al. 1988, Christensen et al. 1993, van Winkelhoff et al. 1993, Contreras & Slots 1996, van Winkelhoff & Slots 1999). The frequency of bacteraemia detection in this study was lower than that quoted by previous workers. However, it is possible that patients with adult periodontitis present a unique patient base whose immune systems are highly primed to cope with periodontal bacteria, so that when a bacteraemia is induced it is quickly and efficiently cleared by the patient's reticuloendothelial system. However, bacteria lodged in sites such as the liver or elsewhere in the system could continue to exert a detrimental effect on the host by inducing high levels of circulating acute-phase proteins.

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