A Clinico–Biochemical Evaluation of the Role of a Herbal (Ayurvedic) Immunomodulator in Chronic Periodontal Disease: A Pilot Study

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Abstract

Background: Host modulation is fast gaining popularity as a preferred therapeutic modality for periodontal disease. Recent research in the medical field into herbal immunomodulators such as Septilin® has spurred an interest in evaluating its efficacy in periodontitis for the first time.

Aim: The aim of the study was to assess the immunomodulatory effects of the herbal immunomodulator Septilin® (Himalaya Drug Company, Bangalore, India) when used as an adjunct to scaling and root planing in chronic periodontal disease.

Methods: Forty systemically healthy patients aged between 25 and 55 years of age and with chronic periodontitis were randomly divided into two groups. The test group was administered Septilin® tablets for two weeks following scaling and root planing whereas the control group was treated by scaling and root planing alone. Changes in gingival index (GI), gingival bleeding index (GBI), serum C-reactive protein (CRP) levels and salivary tumour necrosis factor-alpha (TNF-α) levels were assessed at day 0, at two weeks, and at three and six months.

Results: The GI and GBI showed a statistically significant reduction at two weeks, three months and six months ($P<0.001$) in both groups. Salivary TNF-α level reduction was significant in the test group only ($P<0.001$). No significant change was found in serum CRP levels in both groups ($P>0.05$).

Conclusion: In this pilot evaluation, Septilin® was found to be a safe and effective immunomodulator as an adjunct to routine periodontal therapy. Further long-term studies to test Septilin® on larger sections of the population are recommended.

Key Words: Periodontitis, Herbal Immunomodulation, Serum C-Reactive Protein, Human Tumour Necrosis Factor-α, ELISA, Immunoturbidometry

Introduction

Ayurveda is an ancient Indian science that mainly involves the use of naturally occurring herbs and shrubs to provide a cure for medical ailments without causing any undue side effects. Ayurveda is popular in Asia and Europe and in the opinion of the authors, is fast gaining recognition worldwide. The identification of suitable drugs and preparations from natural sources for preventing immunological complications of various organs is gaining increasing attention [1]. It has been observed that such herbal drugs tend to exert their effect by modulating both humoral and cellular immune functions. Many herbal drugs have shown the capacity to control the production of proinflammatory mediators thereby managing many inflammatory processes [2]. It has been established that the activation of macrophage and other immunocompetent cells plays a major role in the manifestation of inflammation [3]. Among different bacterial antigens, lipopolysaccharide (LPS) is a potent activator of macrophages [4]. LPS is known to evoke a wide range of signalling pathways in macrophage and other cell types leading to the production of inflammatory mediators [5,6]. Such inflammatory mediators consist of proinflammatory cytokines such as tumour necrosis factor-alpha (TNF-α), interleukin (IL) (IL-1, IL-6, IL-8) and other mediators such as nitric oxide (NO) and prostaglandin [7].

Proinflammatory cytokines such as TNF-α, IL-1α, IL-6, IL-8 have been found to be responsi-

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ble for the cellular and tissue damage leading to inflammation [8]. Production of such cytokines from macrophages and other cells by the action of indigenous and exogenous antigens can trigger the initiation of inflammation [9-11]. Some of these inflammatory mediators such as TNF-α also induce hepatic release of certain acute phase proteins such as C-reactive protein (CRP). Several studies have found associations between periodontitis and elevated serum CRP levels [12-14]. Mechanical periodontal therapy has also been shown to reduce CRP levels [15]. Reduction in serum CRP appears to be greater in patients treated with systemic or local antibiotics along with standard periodontal treatment compared to those receiving standard periodontal treatment alone [16,17].

More recently, herbal drugs have been found to be promising in managing inflammatory disorders [18,19]. Septilin® (Himalaya Drug Company, Bangalore, India) is a herbal drug preparation that has shown some potency to modulate immune functions in animal models [20,21]. It includes various herbal products, each with its own unique immunomodulatory effect (*Table 1*). *In vitro* studies have investigated the mechanism of Septilin® in regulating the production of proinflammatory mediators such as TNF-α, IL-6, IL-8, NO, cycloxygenase (COX-2) and phosphodiesterase (PDE4) in LPS-stimulated macrophage and monocyte cell lines [22]. Because these mediators are also actively involved in periodontal disease, it was decided to carry out for the first time as a pilot project the clinical evaluation of the immunomodulatory effects of Septilin® as an adjunct to scaling and root planing on periodontal parameters and on proinflammatory markers, salivary TNF-α and serum CRP.

Table 1. *Composition of a Septilin® tablet*

	Constituent	**Quantity**
Powder	*Balsamodendron mukul*	162 mg
	Shankha bhasma	32 mg
Extract	Maharasnadi quath	65 mg
	Tinospora cordifolia	49 mg
	Rubia cordifolia	32 mg
	Emblica officinalis	16 mg
	Moringa pterygosperma	16 mg
	Glycyrrhiza glabra	6 mg

Aim

The aim of the study was to assess the immunomodulatory effects of herbal immunomodulator Septilin®, when used as an adjunct to scaling and root planing in chronic periodontal disease.

Methods

Following the approval of the Ethical Committee, Bangalore Institute of Dental Sciences, Bangalore, 40 patients aged between 25 and 55 years were recruited during the period January 2011 to April 2011 from the Periodontics Department of the Bangalore Institute of Dental Sciences and Postgraduate Research on the basis of the following criteria:

1. Patients with generalised or localised chronic periodontitis having a probing pocket depth of >5 mm.
2. Patients otherwise systemically healthy.
3. Patients with no history of illness or drug intake in the last six months.

Patients with a compromised immune system, deleterious habits such as smoking, betel nut/paan chewing, pregnant or lactating women, and physically or mentally challenged individuals were excluded from the study.

Fifty-two patients met the inclusion criteria, of whom 44 agreed to take part in the study. However, four did not attend their recall visits during the study period and were excluded. The final sample was therefore 40 patients. They were divided randomly into two groups, each of 20 patients:

Group 1: Test group in which Septilin® tablets were administered twice a day for two weeks following scaling and root planing.

Group 2: Control group in which only scaling and root planing was performed and no medication was administered.

Periodontal parameters—that is, gingival index (GI) [23] and gingival bleeding index (GBI) [24]—were assessed prior to scaling (at day "0") and at two weeks, three months and six months post therapy. Saliva samples for TNF-α analysis were analysed using a human TNF-α ELISA kit (KrishGen Bio Systems, Mumbai, India) and blood samples for serum CRP analysis were collected on day 0 and after two weeks, three months and six months and analysed by immunoturbidimetry.

The operators and examiners in this study were junior postgraduate research students who had been adequately trained in ultrasonic scaling and root planing and the recording of periodontal indices. One postgraduate student was responsible for the recording of the indices pre-operatively and at the different time intervals so as to negate intra-exam-

iner variations. Another postgraduate student was delegated to perform the ultrasonic scaling and root planing for all 40 patients to avoid operator bias.

All patients enrolled for the study were asked to follow the following oral hygiene regimen:

1. Brush your teeth twice daily.
2. Rinse your mouth thoroughly after every meal.

At every monthly recall visit throughout the study, oral prophylaxis was carried out for all the patients as part of their maintenance programme. No specific dietary instructions were given to the patients except that they were asked to continue with the same dietary pattern that they had been following prior to the study.

Data were entered into statistical software (Statistical Package for the Social Sciences Version 13; SPSS Inc, Chicago, IL, USA) and statistical analysis was carried out using the Student's *t*-test, Wilcoxon signed rank test and Mann-Whitney test.

Results

The change in mean GI levels from the pre-treatment (baseline) was found to be statistically significant when compared at two weeks ($P<0.001$), three months ($P<0.001$) and six months ($P<0.001$) within the test and within the control group (*Tables 2A* and *2B*). However, the test group showed a significant difference in mean GI compared to the control group at two weeks ($P<0.01$), three months ($P<0.01$) and six months ($P<0.001$) (*Table 2C*).

The change in mean GBI levels from baseline was found to be statistically significant when compared with the levels at two weeks ($P<0.001$), three months ($P<0.001$) and six months ($P<0.001$) within both the test and control groups (*Tables 3A* and *3B*) whereas the test group showed a significant reduction in the mean GBI when compared to the control group at two weeks ($P<0.001$), three months ($P<0.001$) and six months ($P<0.001$) (*Table 3C*).

The change in mean TNF-α levels from pre-treatment (baseline) was found to be statistically significant when compared with two weeks ($P<0.001$), three months ($P<0.001$) and six months ($P<0.001$) within the test group (*Table 4A*); however, no significant change was observed in mean TNF-α levels from pre-treatment (baseline) to two weeks, three months and six months ($P>0.05$) within the control group and also between the test and control groups at the various time intervals ($P>0.05$) (*Tables 4B* and *4C*).

The change in mean serum CRP levels from pre-treatment (baseline) to other time intervals was not found to be statistically significant within the test group ($P>0.05$) and within the control group ($P>0.05$) (*Tables 5A* and *5B*) and no significant difference was observed between the test and control groups with respect to the mean serum CRP levels at any of the time intervals ($P>0.05$) (*Table 5C*).

Discussion

Ayurvedic medicine is mainly channelled towards regulation of the human immune system for curing various medical disorders without any undesirable side effects. This approach provides a treatment option for a number of immune-related disorders and chronic bacterial infections. Immunomodulatory disease-modifying agents have been used in human medicine for relapsing–remitting immune-mediated diseases [25]. Because the immune response plays a role in periodontal breakdown, it was decided to evaluate the immunomodulatory effects of Septilin® on the disease process for the first time. Immunomodulators alter the activity of immune function through the dynamic regulation of informational molecules such as cytokines. Cytokines, a large group of soluble extracellular proteins or glycoproteins, are key intercellular regulators and mobilisers. It has been suggested that modulation of cytokine secretion may offer novel approaches in the treatment of a variety of diseases [2]. TNF-α is a pro-inflammatory cytokine that induces bone resorption and up-regulates prostaglandin E2 (PGE2) and matrix metalloproteinase (MMP) secretion. Its role in periodontal disease is also well documented [26]. TNF-α is also known to induce the expression of other inflammatory mediators that potentiate inflammatory responses, such as the prostaglandins and MMPs [27,28]. In 2001, in a major breakthrough as far as host modulation is concerned, an antagonist to TNF, a pivotal cytokine in the pathogenesis of rheumatoid arthritis (RA), was discovered. It was one of the most important advances in RA treatment [29].

The acute phase response is a non-specific process that may occur in the initial host response to periodontal infection. It is initiated by the activation of local macrophages and other cells (including fibroblasts and endothelial cells), leading to the release of mediators such as TNF-α, IL-6. These in turn cause systemic changes including hepatic release of a range of plasma proteins such as CRP, activation of complement proteins, and various

Table 2. *Mean gingival index scores in the test and control groups*

A: Comparison of the change in mean GI levels at different time intervals (from baseline) in test group (paired *t*-test)

Time interval	Mean	SD	SE of mean	Mean difference	*t*	*P*-value
Pre-treatment	1.91	0.25	0.06	0.263	5.447	<0.001*
2 weeks	1.65	0.29	0.06			
Pre-treatment	1.91	0.25	0.06	0.345	6.560	<0.001*
3 months	1.56	0.30	0.07			
Pre-treatment	1.91	0.25	0.06	0.391	6.712	<0.001*
6 months	1.52	0.32	0.07			

*denotes significant difference

B: Comparison of the change in GI levels at different time intervals (from baseline) in control group (*t*-test)

Time interval	Mean	SD	SE of mean	Mean difference	*t*	*P*-value
Pre-treatment	2.01	0.27	0.06	0.073	3.899	0.001*
2 weeks	1.94	0.27	0.06			
Pre-treatment	2.01	0.27	0.06	0.117	4.896	<0.001*
3 months	1.90	0.27	0.06			
Pre-treatment	2.01	0.27	0.06	0.134	6.353	<0.001*
6 months	1.88	0.27	0.06			

*denotes significant difference

C: Comparison of GI between test group and control group (*t*-test)

Time interval	Group	Mean	SD	SE of mean	Mean difference	*t*	*P*-value
Pre-treatment	Test	1.91	0.25	0.06	-0.106	-1.271	0.211
	Control	2.01	0.27	0.06			
2 weeks	Test	1.65	0.29	0.06	-0.296	-3.365	0.002*
	Control	1.94	0.27	0.06			
3 months	Test	1.56	0.30	0.07	-0.334	-3.692	0.001*
	Control	1.90	0.27	0.06			
6 months	Test	1.52	0.32	0.07	-0.363	-3.898	<0.001*
	Control	1.88	0.27	0.06			

*denotes significant difference in test group

metabolic changes. CRP and other acute phase molecules are usually present at relatively low levels in plasma, but may be raised dramatically within 72 hours of tissue injury or infection [14]. CRP levels appear after the onset of disease and levels increase within 4 to 6 hours after an acute tissue injury. Consistently high levels of CRP have been found in periodontal infections [30,31]. Increased CRP levels have been associated with a high risk of cardiovascular disorders [32]. Levels of CRP in the range 1-3 mg/L have gained special attention as risk factors for cardiac and cerebrovascular events [14]. The present study found serum CRP levels in both the test and control groups ranging from 0-6 mg/L in the pre-treatment phase, which definitely emphasises the need to reduce the levels of CRP in periodontal disease.

Numerous methods have been adopted to

Table 3. *Mean gingival bleeding index scores in the test and control groups*

A: Comparison of the change in GBI levels at different time intervals (from baseline) in test group (paired *t*-test)

Time interval	Mean	SD	SE of mean	Mean difference	*t*	*P*-value
Pre-treatment	0.93	0.10	0.02	0.222	10.306	<0.001*
2 weeks	0.71	0.10	0.02			
Pre-treatment	0.93	0.10	0.02	0.254	11.757	<0.001*
3 months	0.68	0.10	0.02			
Pre-treatment	0.93	0.10	0.02	0.287	12.990	<0.001*
6 months	0.65	0.10	0.02			

*denotes significant difference

B: Comparison of the change in GBI levels at different time intervals (from baseline) in control group (paired *t*-test)

Time interval	Mean	SD	SE of mean	Mean difference	*t*	*P*-value
Pre-treatment	0.98	0.04	0.01	0.124	11.513	<0.001*
2 weeks	0.86	0.04	0.01			
Pre-treatment	0.98	0.04	0.01	0.144	13.370	<0.001*
3 months	0.84	0.05	0.01			
Pre-treatment	0.98	0.04	0.01	0.166	15.329	<0.001*
6 months	0.81	0.05	0.01			

*denotes significant difference

C: Comparison of GBI between test group and control group (*t*-test)

Time interval	Group	Mean	SD	SE of mean	Mean difference	*t*	*P*-value
Pre-treatment	Test	0.93	0.10	0.02	-0.045	-1.856	0.071
	Control	0.98	0.04	0.01			
2 weeks	Test	0.71	0.10	0.02	-0.143	-5.824	<0.001*
	Control	0.86	0.04	0.01			
3 months	Test	0.68	0.10	0.02	-0.154	-6.353	<0.001*
	Control	0.84	0.05	0.01			
6 months	Test	0.65	0.10	0.02	-0.166	-6.605	<0.001*
	Control	0.81	0.05	0.01			

*denotes significant difference in test group

detect the presence and quantities of these parameters in the serum and other fluids in order to establish a positive link to disease. The most commonly used methods involve the assessment of gingival crevicular fluid (GCF) and saliva. Because these fluids are derived from serum, some molecules present in blood also appear in them [33]. Recently, there has been increasing interest in diagnosis based on saliva analyses, because saliva has a simple and non-invasive collection method. Oral fluid sampling is safe for the operator and the patient, and has easy and low-cost storage [34]. Moreover, increasing evidence indicates that detection of GCF-derived mediators in saliva may serve as a means of rapid screening for periodontal disease [35-37]. In the present study, therefore, it was decided to use saliva as a diagnostic marker to assess levels of TNF-α quantitatively.

Many herbal drugs have shown the capacity to control the production of proinflammatory media-

Table 4: *Salivary TNF-α levels in the test and control groups*

A: Comparison of the change in TNF-α levels at different time intervals (from baseline) in test group (paired *t*-test)

Time interval	Mean	SD	SE of mean	Mean difference	*t*	*P*-value
Pre-treatment	6.74	4.12	0.92	3.706	4.215	<0.001*
2 Weeks	3.03	1.47	0.33			
Pre-treatment	6.74	4.12	0.92	3.877	4.415	<0.001*
3 Months	2.86	1.34	0.30			
Pre-treatment	6.74	4.12	0.92	4.192	4.736	<0.001*
6 Months	2.55	0.91	0.20			

*denotes significant difference

B: Comparison of the change in TNF-α levels at different time intervals (from baseline) in control group (paired *t*-test)

Time interval	Mean	SD	SE of mean	Mean difference	*t*	*P*-value
Pre-treatment	2.55	0.80	0.18	-0.178	-0.673	0.509
2 weeks	2.73	0.97	0.22			
Pre-treatment	2.55	0.80	0.18	-0.237	-0.905	0.377
3 months	2.79	0.96	0.22			
Pre-treatment	2.55	0.80	0.18	-0.134	-0.504	0.620
6 months	2.68	0.92	0.20			

C: Comparison of TNF-α between test group and control group (paired *t*-test))

Time interval	Group	Mean	SD	SE of mean	Mean difference	*t*	*P*-value
Pre-treatment	Test	6.74	4.12	0.92	4.189	4.464	<0.001*
	Control	2.55	0.80	0.18			
2 weeks	Test	3.03	1.47	0.33	0.306	0.779	0.441
	Control	2.73	0.97	0.22			
3 months	Test	2.86	1.34	0.30	0.075	0.205	0.839
	Control	2.79	0.96	0.22			
6 months	Test	2.55	0.91	0.20	-0.136	-0.470	0.641
	Control	2.68	0.92	0.20			

*denotes significant difference

tors thereby managing many inflammatory processes [2]. Long-term use of such herbal anti-inflammatory drugs was found to be safer than chemical anti-inflammatory drugs [38]. Septilin® is an Ayurvedic herbal preparation containing various herbs and minerals. It contains numerous medicinal plants that possess immunomodulatory properties that aid in strengthening the immune system. Septilin® possesses immunomodulatory and anti-inflammatory properties, which potentiate the non-specific immune responses of the body [39,40]. It has been reported to have anti-bacterial, anti-inflammatory, anti-exudative and immunostimulatory effect [41,42]. Studies in India have shown Septilin® to be effective in respiratory tract infections, tonsillitis, and other infections [43-45]. The present study was the first of its kind to assess the efficacy of this herbal preparation in periodontal infections. Because it was a pilot project, it was decided to include only two groups, namely test and control, as the objective was primarily to evaluate whether administration of Septilin® enhanced

Table 5. *Serum CRP levels in the test and control groups*

A: Comparison of the change in serum CRP levels at different time intervals (from baseline) in test group (paired *t*-test)

Time interval	Mean	SD	SE of mean	Mean difference	*t*	*P*-value
Pre-treatment	1.72	0.98	0.22	0.112	0.398	0.695
2 weeks	1.61	1.56	0.35			
Pre-treatment	1.72	0.98	0.22	0.332	1.690	0.107
3 months	1.39	1.08	0.24			
Pre-treatment	1.72	0.98	0.22	0.395	2.095	0.050
6 months	1.33	1.07	0.24			

B: Comparison of the change in serum CRP levels at different time intervals (from baseline) in control group (paired *t*-test)

Time interval	Mean	SD	SE of mean	Mean difference	*t*	*P*-value
Pre-treatment	1.53	1.38	0.31	0.086	0.511	0.615
2 weeks	1.44	1.14	0.25			
Pre-treatment	1.53	1.38	0.31	0.012	0.072	0.943
3 months	1.52	1.11	0.25			
Pre-treatment	1.53	1.38	0.31	-0.004	-0.019	0.985
6 months	1.53	1.13	0.25			

C: Comparison of serum CRP levels between test group and control group (paired *t*-test)

Time interval	Group	Mean	SD	SE of mean	Mean difference	*t*	*P*-value
Pre-treatment	Test	1.72	0.98	0.22	0.193	0.509	0.604
	Control	1.53	1.38	0.31			
2 weeks	Test	1.61	1.56	0.35	0.167	0.387	0.701
	Control	1.44	1.14	0.25			
3 months	Test	1.39	1.08	0.24	-0.128	-0.367	0.716
	Control	1.52	1.11	0.25			
6 months	Test	1.33	1.07	0.24	-0.206	-0.589	0.559
	Control	1.53	1.13	0.25			

the routine therapeutic outcomes in periodontal disease. It was decided not to include administration of a placebo to the control group because recent research protocols suggest this practice to be unethical [46,47]. No patient reported any untoward and unpleasant reactions to Septilin®, indicating it to be a relatively safe drug for use.

The test group, which was administered Septilin®, showed significant improvement in gingival inflammation and bleeding, together with salivary TNF and serum CRP during the various time intervals, which confirmed existing evidence justifying anti-inflammatory effects of Septilin® [21,39,45]. Significant improvement was also seen in the control group during various time intervals. This is in accordance with previous studies that have concluded that gingival health can be restored and maintained by a combination of effective oral hygiene maintenance and non-surgical operative procedures [48-50]. A pocket probing depth of ≥5 mm was only used as a selection criterion. It was not used as a parameter for measuring treatment outcomes because the aim of this pilot study was to assess immunomodulatory effects that, in the opinion of the authors, are clinically reflected by parameters such as gingival inflammation and bleeding and biochemical markers such as salivary TNF and serum CRP. However, gingival inflammation, as

evidenced by the gingival index and bleeding assessment, showed more significant reduction in the test group when compared to the control group where no drug was administered. This further confirms the anti-inflammatory effects of Septilin® as both clinical and laboratory studies have shown that it possesses anti-inflammatory properties [39,45]. Septilin® has also exhibited COX-2 enzyme inhibitory activity using *in vitro* screening kit and has been shown to down-regulate COX-2 gene expression [21]. Various workers have reported herbal drugs with COX-2 inhibiting activity as an alternative to non-steroidal anti-inflammatory drugs (NSAID) for chronic inflammatory conditions [51]. On the other hand, scaling and root planing procedures are mainly targeted towards removal of local factors alone and have no direct effect on the inflammatory mediators [52] whereas host modulation therapies have been developed and proposed to block pathways responsible for periodontal tissue destruction [53]. This possibly explains the reason why the test group showed better improvement than the control group with respect to the clinical parameters.

Furthermore, a significant reduction in TNF-α levels was observed at various time intervals within the test group, which was administered Septilin®, whereas no such change was observed within the control group, which is in accordance with studies stating that herbal preparations have been shown to block TNF-α secretion thereby enhancing anti-inflammatory effect and reducing tissue destruction [21,51,54]. This further emphasises the role of scaling and root planing not having a direct effect on the inflammatory markers. Because of this, the control group did not show any significant change in the TNF-α levels from pre-treatment levels [52].

There was no significant difference in the reduction in the serum CRP levels postoperatively, within both the test group and the control group. CRP is an abnormal protein that appears in the serum only during the acute phase of inflammatory diseases of infectious or non-infectious origin [55-57]. The amount decreases and eventually disappears with the subsidence of the disease process and the recovery of the patient [58,59]. This could possibly explain why changes in CRP levels did not show significant changes in both the groups.

Because Septilin® is a relatively new drug for the treatment of periodontal disease, the current study was a pilot project, hence a small sample size, Further studies with a larger sample size and comparisons with other effective and proven immunomodulators are the obvious next step. In addition, the use of a more sensitive diagnostic fluid such as GCF to assess the levels of the disease markers is recommended. Such a study is currently under way.

Conclusion

The results of this pilot project suggest that Septilin® (a herbal immunomodulatory agent) is a promising adjunct to scaling and root planing which may aid in improving periodontal treatment outcomes because the drug is safe and has shown beneficial effects on the clinical and biologic markers of periodontal disease. The results warrant long-term studies on a larger population.

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Contributions of each author

- SSh: principal coordinator of the study, mainly responsible for study design, coordinating with Himalaya drug company, and supervising study overall with regard to selection/approval of cases and execution of the study. Responsible for preparation of manuscript.
- AB: principal investigator, examined all the study cases, supervised recording of clinical findings, and solely responsible for patient-related aspects.
- SSr: additional coordinator between investigator and the chief coordinator; also supervised clinical part of the study with the investigator.
- AS: coordinator for biochemical aspect of study between the investigator and laboratory at the Himalaya Drug Company.

Ensured proper collection of the blood and saliva samples from patients and their subsequent analysis.

- AR: Provided guidance and support. Coordinated with Ethical Committee.

Statement of conflict of interest

Other than the fact that the manufacturers of Septilin® funded this study, the authors are aware of no other potential or actual conflict of interests.

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