Reviewer's report

Title: Prevalence of systemic immunoreactivity to Aggregatibacter actinomycetemcomitans leukotoxin in relation to the incidence of myocardial infarction

Version: 1 Date: 22 October 2010

Reviewer: keith mintz

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It is also widely accepted that inflammation and the associated signaling molecules may play a role in CVD. Hence, inflammatory diseases, such as periodontal diseases may in fact contribute, at some level, to CVD. However, the mechanism(s) for this relationship has not been determined, to this reviewer's knowledge. This group has demonstrated that the leukotoxin of Aggregatibacter actinomycetemcomitans stimulates the release of IL-1β from macrophages and therefore, proposes the interesting hypothesis that leukotoxin may be a marker or a factor in the development of CVD. They have recently determined a negative correlation between leukotoxin and stroke. In this study, a negative correlation between the presence of leukotoxin and MI was determined.

A major concern of the study is the lack of any microbiological data on the incidence of A. actinomycetemcomitans in this cohort. In addition, the high number of individuals with neutralizing leukotoxin antibodies in relation to the incidence of A. actinomycetemcomitans and periodontal disease in this population raises questions about the assay.

Specific concerns and questions:

1. The study is based on neutralization of leukotoxin activity by plasma samples containing neutralizing antibodies. Plasma or a dilution of plasma is mixed with a fixed amount of purified leukotoxin and exposed to leukocytes. Leukocyte killing is determined by the amount of lactate dehydrogenase released from the dead or dying cells. Was the amount of LDH determined in the individual samples before addition to the leukocytes? Since LDH is found in all tissues and RBCs, levels may vary due to the amount of RBC lysis during phlebotomy or other disease factors. What is the average amount of LDH in plasma? How does this value compare to the release of LDH from the leukocytes in this assay?

2. In terms of high and low individuals is this an avidity or an affinity difference?

3. Instead of using whole plasma, has the antibody fraction of these individuals been isolated and shown to directly interact with isolated leukotoxin?

4. Have add-back studies been performed with null and high level plasmas?

5. The hypothesis of the paper clearly states a virgin infection of A. actinomycetemcomitans may be involved. Isolation of the bacteria from the oral cavity or the blood would be much more convincing than stating that “the periodontal status is not known but is expected into be in line with a similar
recently examined Swedish population (26).

6. The finding in this study suggests over half of the MI cohort has neutralizing antibodies. Yet, the presence of A. actinomycetemcomitans in the population of either healthy or diseased individuals is much less. How does one account for this difference? Have these plasma samples been tested for reactivity to the whole bacteria?

7. Are IL-1# levels increased in the serum of the individuals with increased antibodies?

**Level of interest:** An article of limited interest

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests