Supplemental information for:

**Exposure to *Aggregatibacter actinomycetemcomitans* before symptom onset and the risk of evolving to rheumatoid arthritis**

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**Table S1.** Descriptive data of pre-symptomatic individuals, early RA, established RA controls and periodontitis healthy individuals.

| | Pre-sympt.i. (n=526) | *P | Early RA (n=231) | *P | Established RA (n=168) | *P | Control (n=567) | PH (n=73) |
|---|---|---|---|---|---|---|---|---|
| Women, n (%) | 375 (71.3) | 0.10 | 159 (68.8) | 0.55 | 111 (66.1) | 0.88 | 378 (66.7) | 45 (61.6) |
| Age, years Mean±SD | 52.2 ± 9.5 | - | 60.2 ± 9.8 | <0.001 | 63.4 ± 11.6 | <0.001 | 52.1 ± 0.4 | 46.8 ± 1.1 |
| Smoker, n (%) | 332/525 (63.2) | <0.001 | 157/230 (68.3) | <0.001 | 109/162 (67.3) | <0.001 | 218/533 (40.9) | - |
| HLA-SE, n (%) | 332/503 (66.0) | 0.03 | 153/227 (67.4) | 0.03 | 108/164 (65.9) | 0.06 | 40/75 (53.3) | - |
| Anti-CCP, n (%) | 201/526 (38.2) | <0.001 | 182/231 (78.8) | <0.001 | 128/168 (76.2) | <0.001 | 23/566 (4.2) | - |
| RF, n (%) | 171/507 (33.7) | <0.001 | 179/231 (77.5) | <0.001 | 140/164 (84.8) | <0.001 | 23/248 (9.3) | - |

*vs. Control. Pre-sympt.i., pre-symptomatic individuals; PH, periodontitis healthy individuals; SE, HLA-shared epitope; anti-CCP, anti-cyclic-citrullinated peptide antibody positive individuals; RF, rheumatoid factor positive subjects.
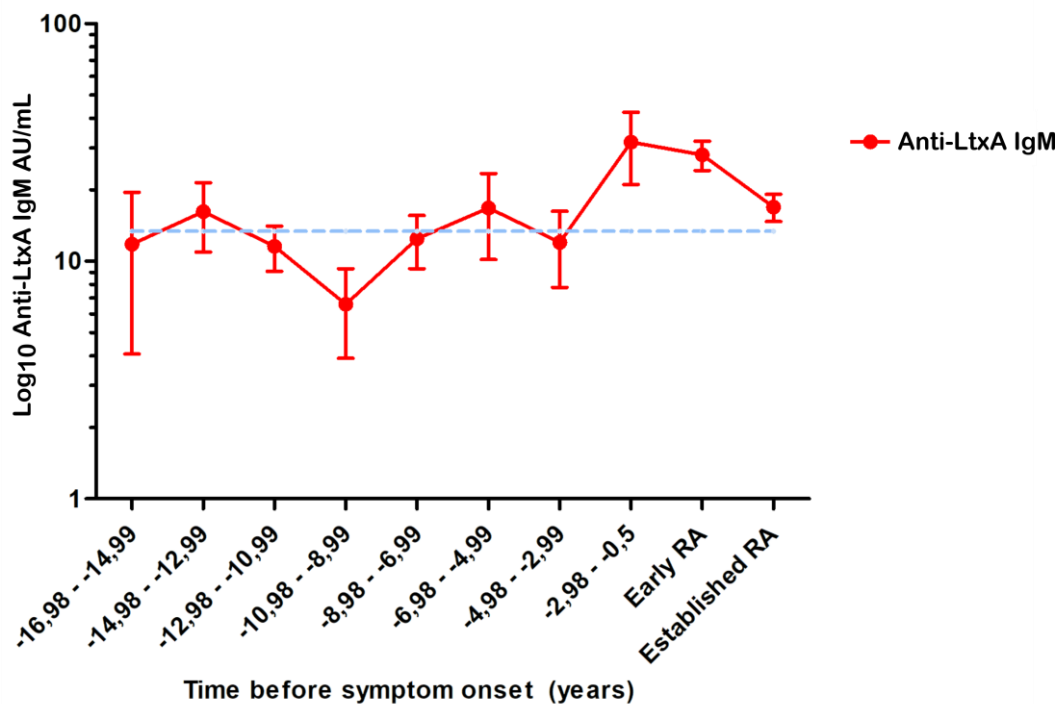


**Figure S1.** Trajectory of Anti-LtxA IgM antibody levels in pre-symptomatic individuals. **Log10** mean (red dots) and standard error of the mean (error bars) of anti-LtxA IgM levels (AU/mL) for the same individuals during the predating time until symptom onset as pre-symptomatic individuals (n = 231), as patients with early RA (n=231) and with established RA (n=108). The dotted line in blue represents the mean anti-LtxA IgM levels of non-RA controls (n = 567).

**Table S2.** Anti-LtxA IgA concentration according to demographic factors.

| | | Pre-sympt.i. n=526 | *P | Early RA n=231 | *P | Established RA n=168 | *P | Control n=567 |
|---|---|---|---|---|---|---|---|---|
| **Sex** | Male | 76.2 (14.14) | ns | 100.4(27.06) | ns | 70.2(19.93) | ns | 94.8(19.19) |
| | Female | 88.8(15.5) | ns | 83.4(17.18) | ns | 88.0(17.78) | ns | 124.9(14.67) |
| **Smoker** | Ever | 65.7(9.61) | ns | 79.4(17.56) | ns | 67.2(14.04) | ns | 76.0 (15.05) |
| | Never | 116.8(27.32) | ns | 107.7(26.08) | ns | 101.8(29.45) | ns | 141.9(16.89) |
| **RF** | Positive | 82.6(12.95) | ns | 74.7(13.81) | ns | 84.0(15.16) | ns | 68.2(29.2) |
| | Negative | 83.5(16.83) | ns | 136.9(43.17) | ns | 75.6(33.19) | ns | 130.9(21.77) |
| **Anti-CCP** | Positive | 75.7(11.86) | ns | 90.3(16.68) | ns | 90.8(16.54) | ns | 157.5(56.87) |
| | Negative | 91.2(17.59) | ns | 83.0(29.25) | ns | 53.6(20.47) | ns | 113.3(11.97) |
| **HLA-SE** | Positive | 74.5(10.3) | ns | 82.3(18.01) | ns | 69.4(14.41) | ns | 122.2(38.57) |
| | Negative | 112.1(29.98) | ns | 100.6(25.37) | ns | 109.9(29.42) | ns | 134.8 (43.35) |

Pre-sympt.i., pre-symptomatic individuals; HLA-SE, HLA-shared epitope; anti-CCP, anti-cyclic-citrullinated antibody positive individuals; RF, rheumatoid factor subjects. * *vs*. Control, Values are represented as mean (SEM).

**Table S3.** Anti-LtxA IgG concentration according to demographic factors.

| | | Pre-symp.i. n=526 | *P | Early RA n=231 | *P | Established RA n=168 | *P | Control n=567 |
|---|---|---|---|---|---|---|---|---|
| **Sex** | Male | 111.3(14.81) | ns | 135.6(27.8) | ns | 98.9(25.59) | ns | 101.9(14.02) |
| | Female | 100.8(9.2) | ns | 118.9(17.2) | ns | 112.3(18.66) | ns | 131.6(11.94) |
| **Smoker** | Ever | 89.9(8.65) | ns | 105.9(15.01) | ns | 101.6(18.67) | ns | 95.4(12.3) |
| | Never | 121.1(13.61) | ns | 152.7(31.45) | ns | 114.4(25.77) | ns | 143.6(13.89) |
| **RF** | Positive | 113.5(13.83) | ns | 126.8(16.62) | ns | 109.9(16.34) | ns | 49.9(13.72) |
| | Negative | 97.1(9.67) | ns | 114.6(31.25) | ns | 97.6(43.51) | ns | 130.5(14.45) |
| **Anti-CCP** | Positive | 106.1(12.11) | ns | 131.0(17.54) | ns | 111.3(16.49) | ns | 133.4(38.72) |
| | Negative | 102.4(10.2) | ns | 98.0(22.85) | ns | 96.3(35.08) | ns | 121.4(9.51) |
| **HLA-SE** | Positive | 106.44(10.2) | ns | 108.58(16.61) | ns | 89.34(16.04) | ns | 145.42(45.24) |
| | Negative | 104.34(13.17) | ns | 155.21(29.47) | ns | 146.13(32.39) | ns | 168.22(40.45) |

* *vs*. Control. Pre-sympt.i., pre-symptomatic individuals; HLA-SE, HLA-shared epitope; anti-CCP, anti-cyclic-citrullinated peptide antibody; RF, rheumatoid factor. * *vs.* Control, Values are represented as mean (SEM).

**Table S4.** Predictive value of anti-LtxA IgM positivity for development of RA.

**Pre-sympt.i.**

| Independent variable | Unadjusted OR (95% CI) | Adjusted OR (95% CI) | | | | |
|---|---|---|---|---|---|---|
| Anti-LtxA IgM (+) | 1.658 (0.954, 2.883) | 1.504 (0.847, 2.671) | 1.418 (0.766, 2.626) | 1.322 (0.598, 2.922) | 5.113 (0.690, 38.181) | 1.757 (1.006, 3.069) *P* = 0.048 |
| Ever Smoker | | 2.467 (1.926, 3.162) | | | | |
| Anti-CCP | | | 14.492 (9.213, 22.797) | | | |
| RF | | | | 4.900 (3.066, 7.829) | | |
| HLA-SE | | | | | 1.690 (1.034, 2.762) | |
| Sex | | | | | | 1.279 (0.986, 1.658) |

**Early RA**

| Independent variable | Unadjusted OR (95% CI) | Adjusted OR (95% CI) | | | | |
|---|---|---|---|---|---|---|
| Anti-LtxA IgM (+) | 3.556 (1.997, 6.334) *P* < 0.001 | 3.051 (1.667, 5.583) *P* < 0.001 | 2.477 (0.986, 5.224) | 3.009 (1.113, 8.133) *P* = 0.030 | 11.133 (1.480, 83.718) *P* = 0.019 | 3.768 (2.097, 6.772) *P* < 0.001 |
| Ever Smoker | | 2.982 (2.144, 4.149 | | | | |
| Anti-CCP | | | 85.137 (50.382, 145.868) | | | |
| RF | | | | 32.834 (19.298, 55.862) | | |
| HLA-SE | | | | | 1.884 (1.096, 3.237) | |
| Sex | | | | | | 1.237 (0.881, 1.737) |

**Established RA**

| Independent variable | Unadjusted OR (95% CI) | Adjusted OR (95% CI) | | | | |
|---|---|---|---|---|---|---|
| Anti-LtxA IgM (+) | 0.999 (0.999, 1.000) | 1.000 (0.999, 1.000) | 0.999 (0.997, 1.000) | 1.000 (0.998, 1.001) | 0.999 (0.998, 1.000) | 0.999 (0.999, 1.000) |
| Ever Smoker | | 2.911 (2.006, 4.219) | | | | |
| Anti-CCP | | | 80.231 (45.675, 140.193) | | | |
| RF | | | | 54.244 (29.361, 99.300) | | |
| HLA-SE | | | | | 1.595 (0.912, 2.792) | |
| Sex | | | | | | 0.988 (0.686, 1.424) |

Pre-sympt.i,, pre-symptomatic individuals; OR, odds ratio; CI, confidence interval; anti-CCP, anti-cyclic-citrullinated peptide antibodies; RF, rheumatoid factor; HLA-SE, Human leukocyte antigen 'shared epitope'. The OR were calculated using logistic regression analyses with the stage of RA development (i.e. pre-symptomatic individuals, early RA, and established RA) as dependent variable. Anti-LtxA IgM was entered as categorical variable.